ФИЗИОЛОГИЯ РАСТЕНИЙ

A Translation Of

PLANT PHYSIOLOGY

(FIZIOLOGIIA RASTENII)

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PLANT PHYSIOLOGY

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CONTENTS

PHYSIOLOGICAL PECULIARITIES OF CALLUS FORMATION BY SECONDARY MERISTEMS

A. M. Siniukhin

Department of Biophysics, N. V. Lomonosov Moscow State University, Moscow

The cells of the primary and secondary meristems are one of the main structural elements which participate directly in the morphological processes of plant organisms. Information about the physiological peculiarities of growth and development in meristematic tissues is very important for the analysis of regeneration in plants. Frequently regeneration processes which occur in the already formed organism as the result of the isolation of removed organs result in either the regeneration of the separated parts or else their replacement by other structures only after the appearance of internal meristematic centers. The success in the use of biologically active substances and many combinations of agricultural measures for stimulating plant regeneration on a large production scale depends, to a large measure, either on the activation of the primary meristem or else the initiation and differentiation of the secondary meristem. However, there is very little experimental data concerning the physiological conditions of the intracellular reactions of the meristematic tissues.

The present paper contains the results of some experiments with plant tissue culture of calluses in which the conditions at which the initiation of secondary growing points in the stem occur were investigated.

Tomato varieties Shtambovyi Alpateva No. 0905A and Luchshii iz vsekh No. 318 were used as experimental material. The experiments were done during 1954-1955.

In order to get callus tissue on the tomato stem, the top of the plant was cut off just above a specific leaf. At the same time all the axillary buds were removed. Calluses were obtained over the first, third, fifth and ninth leaves. As it was demonstrated later on, one can use callus tissue from any stem height in tissue culture. Since the calluses from tomato variety Shtambovyi Alpateva grew slowly at our experimental conditions, and since there was considerable selection after each transfer, most of the experiments were done only with tissue cultures from tomato variety Luchshii iz vsekh (best of all).

The calluses were planted in Ehrlenmyer flasks which were 50 cm^3 in volume. The nutrient medium was prepared according to the method described by White [1].

TABLE 1

Components	Concentration, M	Components	Concentration, M
$MgSO_4$	3	$Fe_2(SO_4)_3$	6
$Ca(NO_3)_2$	1.2	$MnSO_4$	3.7
Na_2SO_4	1.4	$ZnSO_4$	9.4
KNO_3	8	H_3BO_3	1.2
KCl	9	KI	4.5
$NaH_2PO_4 \cdot H_2O$	9	Sucrose	6

All the components of the mixture, with the exception of iron and sucrose, were prepared as concentrated solutions which were kept during the entire experiment. Then a stock solution containing $Ca(NO_3)_2$, Na_2SO_4, KCl, KNO_3, NaH_2PO_4, $MnSO_4$, $ZnSO_4$, H_3BO_3 and KI was prepared by mixing these solutions in a portion of distilled water. During continuous stirring a solution of $MgSO_4$ which had been prepared separately was added to these. In order to prevent the growth of algae, the mixture was kept in a dark bottle. Sucrose and $Fe_2(SO_4)_3$ solutions were prepared anew each time. Only pure reagents were used for culturing the callus tissue.

An extract of brewer's yeast, one of the most suitable mixtures of organic compounds for culturing plant tissues [1] was used in culturing the callus tissue. Ten grains of dry brewer's yeast were mixed in 1 liter of distilled water. The suspension was boiled for 30 min and then centrifuged. The liquid over the sediment was decanted and brought up to a volume of 1 liter. Ten milliliters of extract were added to 1 liter nutrient solution.

The solutions was transferred from the bottle to the flasks with a burette; all the operations were done as quickly as possible. When the flasks had been filled with nutrient solution, they were stoppered with nonhygroscopic cotton covered with gauze. The stoppers were then capped with several layers of parchment paper. The flasks were sterilized for 30 min at one atmosphere. In order to prepare a solid substrate agar was added to the nutrient medium. In order to prevent any possible change in the chemical composition of the nutrient medium, the agar was first very thoroughly washed; 60 g of finely pulverized agar was added to 3 liters distilled water. The water was changed twice a day over a period of one to two weeks. After the agar had been thoroughly washed it was added to the required volume of nutrient solution, filtered, and transferred to flasks. The medium containing the agar was sterilized in an autoclave. When double distilled was used the callus cultures grew considerably better.

Direct sterilization of the callus tissue itself proved most difficult. Mercuric chloride, 85% alcohols and other disinfectants were used for this purpose. Nevertheless, spores of microorganisms which rapidly contaminated the culture frequently remained in the crevices of the rough outer surface of the callus tissue. The percentage of contaminated flasks were as high as 40-60. A lower percentage of contamination (15%) occurred in those cases when the pieces of tissue were first exposed to ultraviolet radiation before their sterilization with chemicals. The results were even better when the entire procedure of transferring the callus tissue cultures was done under ultraviolet light. Contaminated cultures were not counted in the experiment. In one series of the experiment the callus tissue was grown in nutrient solutions at room temperature and in another series in an incubator at 26°. Optimal growth of the callus tissue occurred at the constant temperature.

The formation of calluses in tomato is accompanied by high Eh and rH_2 values. In young callus tissue from tomato variety Shtambovyi Alpateva the Eh value was (+ 150)-(+ 170) mv, and in Luchshii iz vsekh it was (+ 150)-(+ 70) mv; the corresponding rH_2 value of the first was about 15-16, and of the second it was closer to 13 (Fig. 1, A and B).

Since high rH_2 and Eh values are characteristic of young callus tissue, one might conclude that an aerobic type of reaction is characteristic of the cytophysiological processes.

During a colorimetric determination of the oxidation-reduction (abbreviated o-r) potential and the pH_n of the callus tissue in the early stages of formation, an intracellular formation of granules was constantly noticed. The accumulation of granules is also evidence of aerobic processes in the young callus tissue [2-4].

In the final stage of callus tissue development, at a minimum Eh and rH_2 value, and at a minimum hydrogen ion activity (Fig. 1, C), and also at a lowered buffering and o-r capacity of the system (Fig. 2, A and B), the cells change over to processes which are comparatively anaerobic [5]. In tomato callus tissue from Shtambovyi Alpateva the Eh value at this period was (+ 30)-(+ 40) mv, but in the variety Luchshii iz vsekh it was (− 40)-(− 50) mv. The rH_2 value in callus tissue from the first variety was about 13, and in the second one it was close to 10.5. When the rH_2 and pH_n value of the cells at this stage was determined with an indicator, the formation of granules was never observed.

The data obtained (Figs. 1 and 2) indicate that specific stages in the change of cytophysiological processes can be observed during the process of callus tissue formation. The first of these is characterized by a high o-r potential and a low pH_n. The second stage — by maximum values of the o-r potential and an increase in pH_n. The third stage by a minimum o-r potential and maximum pH_n values. The fourth stage by a high o-r potential and low pH_n values.

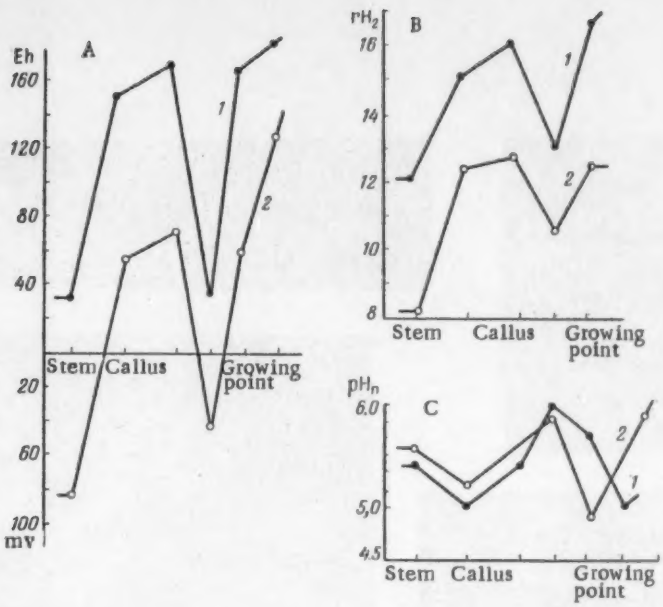

Fig. 1. The change in oxidation-reduction potential (A), rH_2 (B), and hydrogen ion activity (C) during the formation of callus tissue and additional growing points in tomato variety Shtambovyi Alpateva (1) and Luchshii iz vsekh (2).

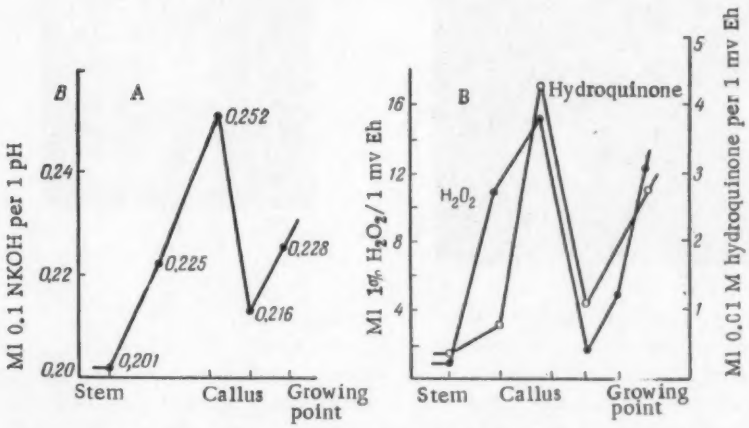

Fig. 2. Buffer capacity (A) and the capacity of the oxidation-reduction system (B) of the callus tissue cells and additional growing points in tomato variety Shtambovyi Alpateva.

The first and second stages are associated with the development of parenchyma cells in the callus tissue; the fourth stage is directly related to the formation and differentiation of secondary meristem centers, and the third stage occurs between the indicated morphological-physiological processes.

On the basis of the importance of the conclusion concerning the necessity of anoerobic conditions for the appearance of secondary meristem centers, we set up several control callus tissue culture experiments.

Calluses in the beginning stages of growth were chosen for culturing in all the variants. The callus was divided into two parts; one of these was transferred to a liquid medium, and the second to a solid nutrient substrata. All the remaining conditions in both variants were identical. The experiments were set up in replicates of 15-20.

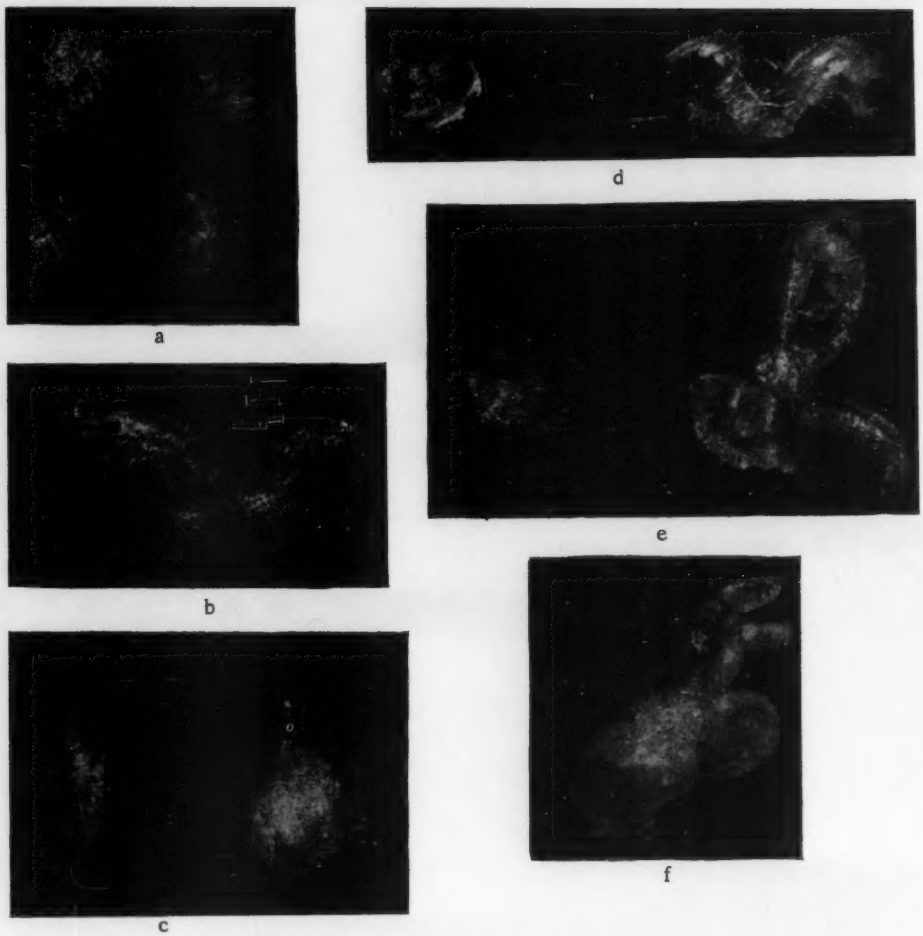

Fig. 3. Cultivation of tissue calluses in tomato variety Luchshii iz vzekh.
a) Growing on an agar surface and b) in liquid nutrient medium, in quadruplicate; c and d) sister cultures of tissue calluses: photograph on the left-callus on solid medium, and on right in White's fluid nutrient medium; calluses in picture d) photographed in fifth week of experiment and e) in sixth week of experiment; f) sister culture of tissue calluse; left) culture tissue on solid medium; right) callus grown in agar medium for the first four weeks and transplanted to fluid nutrient medium on the fifth week; callus photographed at the start of the sixth week; f) culture of tissue callus which was grown on agar surface during the first six weeks and submerged in agar on the seventh week; photographed at the beginning of the tenth week of the experiment.

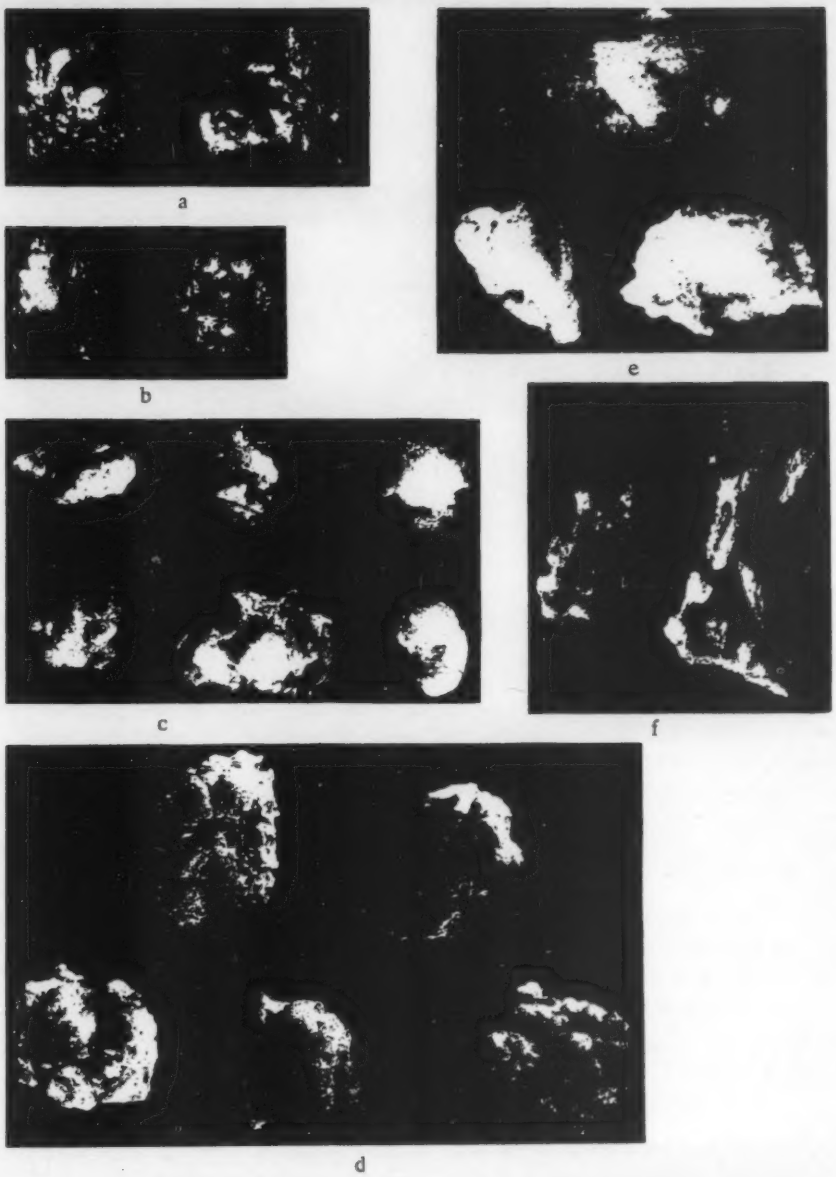

Fig. 4. Culture of tissue callus on tomato variety Luchshii iz vsekh.
a) Sister cultures of tissue calluses grown in White's liquid nutrient medium; b) those cultures grown on White's liquid nutrient with an increased nitrate content; culture photographed on the 14th day of the experiment; c) culture of tissue culture grown on solid agar surface; d and e) calluses grown in liquid nutrient medium with an increased nitrate content; pictures c and d) in the fifth week of the experiment and picture e) at the start of the ninth week; f) sister culture of tissue callus, grown in White's liquid nutrient medium; after development of additional points of germination on callus seen on the right side of the photograph, after transplantation into the medium with a double dosage of nitrate; photographed on the 17th day of the experiment.

The calluses implanted on an agar medium were on the surface of the substrata and were surrounded by air. Under favorable aerobic conditions and with a good supply of nutrients very intensive growth activity was observed. The calluses increased in volume considerably. However, during the four months of growth the formation of secondary growing points was not observed in any of the pieces (Fig. 3, a).

The calluses transferred to a liquid nutrient medium were always submerged in the liquid at the bottom of the Ehrlenmeyer flasks. The height of the liquid nutrient medium in each flask was 15-20 mm. In this variant the calluses grew under comparatively anaerobic conditions. On the calluses which had been submerged in the liquid nutrient medium the initiation of additional growing points began on the third to fifth week. The first leaf which appeared was elongated oval in shape; it consisted of a single leaf blade with an entire margin. The second leaf which developed had three ordinary leaflets; it was trifoliate (Fig. 3, b).

Figure 3, e shows a mass of callus tissue which was transferred to a liquid nutrient medium after it had been growing on agar for four weeks. The callus tissue had enlarged somewhat while it had been growing on the solid agar plate. On the fifth day after the callus tissue had been transferred to the liquid medium the formation of a growing point had begun. On the seventh day a second additional growing point appeared on the same callus culture. Growing points did not develop on the remaining cultures growing on agar.

Significant results were also obtained in the second variant of the experiment. After being transferred to new nutrient medium the tomato callus tissue grew on agar for 6 weeks. During this time under extensive aerobic conditions the cellular mass had increased in volume by 2-2.5 times. Just as in all analogous cases there was no secondary meristem formed. At the beginning of the seventh week the callus tissue which had been on the surface of the agar was pushed down into the nutrient media with a sterile glass rod. Soon after the callus tissue had been submerged within the agar the formation of additional growing points had begun where the cells were definitely under anaerobic conditions (Fig. 3, f).

The following fact can serve as one more indicator that the physiological processes within the cells of the callus tissue with a low rH_2 and Eh value occur anaerobically.

When the callus tissue was grown in a liquid medium containing either a double or a triple dose of nitrates, no secondary meristem was formed. The morphogenesis of the callus tissue in this case ceased just as it did in cultures on agar medium. Figure 4, a shows the sister calluses. In the regular medium two growing points had developed in the callus tissue by the third week after the tissue cultures had been started. However, in the liquid medium with an increased nitrate concentration the formation of secondary meristem and additional growing points had not begun even within the following four weeks (Fig. 4, b).

Figures 4 a, b, and c shows a 5- and 9- week-old callus tissue mass which had grown in a liquid nutrient medium with a dosage of nitrates equal to 2.5 times the regular one. During the culture period the calluses had increased in volume considerably. However, not a single growing point had developed on any of them. Their development had stopped at the first stage in the ontogeny of the callus tissue.

This special feature is characteristic of nitrates; the biological system readily utilizes the oxygen molecules present in them for various oxidation processes including respiration. Nitrate oxygen can replace free oxygen from the air in those cases where the latter either is lacking or present in very small quantities [6,7].

Callus tissue cells located under poorly aerated conditions have a tendency to use the oxygen of endogenous reactions which they obtain from one or another of the components which participate in the anaerobic processes instead of using the inadequate or unavailable supply of external oxygen. Reduction reactions begin to dominate within the callus tissue cells at a decreased partial pressure of oxygen. However, nitrates with their comparatively easily removed oxygen introduced into the nutrient medium in large concentrations considerably change the oxygen relations within the callus tissue cells. Under such conditions the cytophysiological processes occur at the expense of nitrate oxygen instead of at the expense of oxygen removed from one or another of the protoplasmic compounds in which it is more stably bound. This, apparently, explains why the morphogenesis of tomato callus tissue in liquid nutrient medium with an abundance of nitrates proceeds the same as it does in callus tissue cultured under well aerated conditions, i.e. on agar plates.

The growth and development of cells which is characterized by synthesis of materials from carbon whose carbon bonds are made up of methyl and methylene groups to a large degree (amino acids, fatty acids, etc.) is fostered by a decreased oxygen supply. Whereas the formation and differentiation of tissues from simple poly-

saccharides is related to the considerable decrease in the synthetic process of the component parts of the protoplasm and with the increased synthesis of substances, which make up the cell wall, and it occurs more readily under conditions of good aeration [8-14]. On the basis of these conditions, in order to verify the accuracy of the conclusions relative to the experiments with nitrates, one more variant was set up in the tissue culture experiment. Does the differentiation of growing points (but not the initiation of secondary meristem centers) as well as the formation of the cells of the callus tissue mass require aerobic conditions?

White's nutrient medium was poured into the tissue culture flasks. A double portion of nitrates was added to part of them. Calluses which already had small growing point initials were transferred into the flasks. After 16 days of culture two of the sister calluses were photographed (Fig. 4, f). In the regular liquid medium the differentiation of growing points was very slow. The callus tissue did not increase in volume. The formation of additional growing points in the medium with an abundance of nitrates was much more rapid. During this period 3-5 more small leaves had been formed than under the ordinary conditions. At the same time the volume of the callus tissue also increased. The Eh of the additional growing points in Shtambovyi Alpateva was about (+ 165)-(+ 177) mv, but in Luchshii iz vsekh it was equal to + 130 mv. The rH_2 of Shtambovyi Alpateva went up to 17, but in the second variety it was 15 units. During a colorimetric determination of the o-r potential it was always noted that the rH_2 of the cells in the zone of differentiated growing points in Luchshii iz vsekh reached a value greater than 16.0 (Fig. 1). The cells in the differentiated zone of the additional growing points were observed to contain granules when they were stained with an indicator in tomato Shtambovyi Alpateva as well as Luchshii iz vsekh.

The data obtained agree completely with information found in the literature concerning this question. The investigations of Tauson and his co-workers[8, 15] concerning the conditions of callus tissue formation in krymsaghyz showed that the formation of callus tissue as well as the differentiation of growing points occurs only under highly aerated conditions. Partial or complete anaerobiosis retards or completely inhibits the formation of callus tissue and the differentiation of regenerative areas. However, the first stage in the growth of the shoots characterized by meristematic activity, is related to the decrease of the o-r potential [16]. According to the data of Ruhland and Ramshorn [17], the meristematic cells of the root tips and cambia of various woody plants undergo "aerobic fermentation" Cambium cells were found to contain products of alcoholic fermentation, as well as a high respiratory coefficient. The utilization of oxygen in the meristematic cells was considerably lower than it was in the cells which were in the enlarging stage. During the stimulation of the division of the meristematic tissues as the result of their treatment with heteroauxin the accumulation of products resulting from alcoholic fermentation increased. The buds of many trees start to grow sooner at a decreased partial pressure of oxygen [18]. Smirnov [19] found that the respiratory coefficient of dry seeds was greater than one; the author considered that this was due to respiration of embryonic tissue. A low o-r potential is characteristic of animal embryologic tissue [20]. In the regenerating ends of earthworms where the synthesis of protein is high a low Eh has been observed [2]. When regeneration is stimulated it is accompanied by an even greater decrease in o-r potential [22].

An investigation concerning the nature of metabolic process in meristematic tissues was carried out by Turkova [23-26]. On the basis of a study of the oxidation system, taking into account the unique features of biological reactions, the author concluded, that the synthesis of proteinaceous material and the growth phase associated with this is not conducive to an intensive activity of the oxidation processes. On the other hand, they occur more readily at comparatively moderate rates of oxygen respiration and at a highly reducing activity of the tissues. An intensive use of large quantities of oxygen is disadvantageous for the growth of meristems [23-26].

The effect of the partial pressure of oxygen on the formation of additional growing points under the conditions of tissue culture was first demonstrated by White [1, 27]; in this investigation we repeated part of his experiments. In White's experiments, if tobacco stem callus tissue was cultured on solid nutrient medium no growing points were formed after 20 weeks. Under conditions of adequate aeration only those processes associated with the increase in size of the cellular mass were observed. Two weeks after the callus tissue was submerged in a liquid nutrient medium or else in the agar, i.e. when the oxygen supply was inadequate, the development of secondary meristems and the formation of additional growing points began.

SUMMARY

Data found in the literature and those we have received experimentally showed that a high intensity of gas exchange was necessary for the formation of callus tissue. A differentiation of growth points also occurred only when the access of oxygen was most favorable.

With a decrease in the gas exchange of the callus tissues at different stages of initiation of adventious buds differentiation ceased and tumorous leaf and scale-like organs were formed.

The formation of the secondary meristems took place under relatively anaerobic conditions.

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MANGANESE CONTENT OF POLAR PARTS OF WHEAT AND CORN ORGANS

P. A. Vlasiuk and L. D. Lendenskaia

Institute of Plant Physiology, Ukrainian Academy of Sciences, Kiev

A study of the principle of interrelationship between a plant organism and the external environment has shown that the rate of plant growth and development depends on a combination of conditions, expecially on the water supply, the supply of the basic nutrients, and the microelements. A study of the entrance and distribution of the nutrient elements within the plant is very important for explaining their physiological role in the plants.

Investigations on the translocation and distribution of nutrient elements in the plants are greatly concerned with explaining the role of the microelement manganese. An increase in the activity of several of the fermentation systems and vitamin-forming processes by manganese indicates its importance in the process of metabolism and increase of the productivity of agricultural crop plants.

Polarity, the capacity of plants to develop tissues with different characteristics at the morphologically opposite ends of stems, leaves and shoots, determines the direction and the intensity of the metabolic processes. As a result of this the absorption and assimilation of one or another of the nutrient elements varies in the different polar parts of the plant organs and tissues.

In substantiating the theory concerning the stage development of plants Lysenko [1] called attention to the fact that the cells and tissues along the length of the stem have different properties. The tissues of the lower part of the stem are in a younger developmental stage. The upper parts of the stem, the youngest in terms of growth can form the more mature stages of growth.

According to the data of Molotkovskii and Volkoslavskaia [2], the water content of the lower levels of corn, sorghum, cotton and buckwheat was higher than that of the upper ones. The distribution of solids was just the opposite. The increase in content of solids occurred gradually from internode to internode. As the authors showed, the metamers (stem internodes), since they are polar and qualitatively different in formation, are an expression of the stems different qualities. The data obtained by Lopushanskii and Molotkovskii [3] concerning the relationship of ascorbic acid in black walnut where the ascorbic acid content in the seedlings increased from the base to the top should be noted. The upper part of each internode contained more ascorbic acid than the lower part. Turkova's investigations [4] established the rule that the higher the leaf and internode on the stem, the stronger the reducing capacity of the tissues. In internodes of wheat stems, the higher the internode the greater the iodine reducing activity, the higher the ascorbic acid content, and the greater the activity of peroxidase in the stem tissues. It was shown that the reducing agents induced bending of the leaves and stems away from the vertical, whereas, the oxidizing agents were conducive to the vertical position. Hence potassium permanganate and other manganese compounds which one of us has used since 1925-1956 in the nutrient supply to the plants was found to be conducive to the decrease of layering in cereals [5, 6]. Abutalybov [7] showed that most of the mobile forms of manganese are water soluble and are absorbed by the colloidal particles of the growing cotton cells present in the young upper leaves. As the plants become older the amount of these forms of manganese decreases, while at the same time the content of acid soluble manganese increases. Sabinin [8] also stressed the importance of studying the distribution of the individual elements of the plant nutrients in the parts of the branches since an increase in the absorption and accumulation of the elements of mineral nutrition is characteristic of the organs where the biosynthetic processes occur quite intensively.

TABLE 1

The Distribution of Manganese in Parts of Stems from Winter Wheat at Various Conditions of Nutrition (in % of dry weight)

System of nutrition	Internodes from bottom to top					
	first	second	third	fourth	fifth	sixth
Organic-mineral (control)	1.28×10^{-3}	1.68×10^{-3}	1.89×10^{-3}	2.07×10^{-3}	3.59×10^{-3}	4.44×10^{-3}
Organic-mineral + manganese slag	2.25×10^{-3}	2.35×10^{-3}	2.40×10^{-3}	2.48×10^{-3}	2.57×10^{-3}	4.66×10^{-3}

TABLE 2

Polarity Coefficient and the Gradient Coefficient in Polar Parts of Stems from Winter Wheat at Various Conditions of Nutrition

Nutrition system	Polarity coefficient	Gradient coefficient of the internodes				
		from second to first	from third to second	from fourth to third	from fifth to fourth	from sixth to fifth
Organic-mineral (control)	3.46	1.31	1.01	1.22	1.73	1.21
Organic-mineral + manganese slag	2.07	1.04	1.02	1.03	1.04	1.81

Our investigations performed in 1954 [6] showed that the lower, upper, and central parts of the crown of fruit trees differ considerably from each other in their content of the microelement manganese.

In view of the fact that the rate of manganese translocation in plants is limited since insoluble manganese compounds and those which are adsorbed are formed along the way, individual parts of the plant can suffer from an inadequate supply of manganese in spite of the fact that the amount present in the soil may be adequate.

With the object of studying the nature of manganese entrance from the soil solution, its distribution, and its fixation by individual parts of winter wheat and corn plant organs we performed some special investigations during 1955-1956.

The amount of manganese in the plant ash was determined colorimetrically.

In winter wheat plants grown under field conditions in meadow- chernozem- podzolic soil (in replicates of four on plots 100 m^2) the manganese content of the metameric formations (stem internodes) was determined in relation to the polar differentiation.

The results of these determinations are given in Tables 1 and 2.

As one can see from the data in Table 1, the highest manganese content was found in the very youngest sixth stem internode of winter wheat. The increase in the total amount of manganese, especially in plants which had manganese added to the soil, occurred gradually from internode to internode (Table 2). The qualitative difference between two adjacent internodes can be used to estimate the gradient coefficient. The data concerning the dry weight content of walnut stem, obtained by Molotkovskii [9], where the dry weight increased from the top to base, can also be used in the same manner.

As Uspenskii [10] noted, the movement of manganese from old to younger organs is closely related to the slow entrance of manganses into the subterranean part of the plant. According to Vlasiuk and Porutskii [6], the content of exchangeable forms of manganese at the different levels of the cotton stem from the base to the top increased steadily. Romney and Toths [11], using labelled atoms, showed that the manganese content of the

TABLE 3

The Distribution of Manganese in Winter Wheat Plants at Various Nutrient Conditions (in % dry weight)

System of nutrition (in % dry weight)	Root	Stem	Axillary buds	Leaf sheath	Leaves	Stalk of spike	Spike scales	Flower scales	Grain
Organic-mineral	7.87×10^{-3}	2.46×10^{-3}	5.98×10^{-3}	5.66×10^{-3}	1.42×10^{-2}	8.24×10^{-3}	4.69×10^{-3}	4.81×10^{-3}	5.18×10^{-3}
Organic-mineral + manganese slag	1.22×10^{-2}	2.77×10^{-3}	6.50×10^{-3}	6.10×10^{-3}	1.58×10^{-2}	8.64×10^{-3}	8.67×10^{-3}	9.08×10^{-3}	6.75×10^{-3}

apical portions of sunflower, soybean, and buckwheat stems was greater than it was in the lower parts. The apical part of potato tubers, according to Coic and Baisse [12], is richer in manganese than the basal part.

The data we obtained concerning the manganese content of winter wheat is given in Tables 3 and 4.

From Table 3 we can see that the greatest manganese content was found to be in the leaves, and then in the roots. From the roots manganese moves into the aerial organs and sometimes, because of desorption from the secretions, it can get into the soil.

In the plant it can be combined as a salt: tannins, oxalates, etc., and it can also be adsorbed by proteinaceous compounds. Manganese absorbed by the plant can easily be replaced by other cations. We found only traces of manganese in the proteinaceous compounds isolated from plants. An insignificant amount was found in extracts obtained to determine the chlorophyll content of the leaves.

According to Uspenskii's data [10], plants cells rich in protein absorb manganese especially rapidly. It was also shown that the distribution of manganese was related not only to the physiological function of the organs, but also depended on external environmental conditions.

As one can see from the data in Table 3, there was relatively more manganese in the stalks of the spikes and the spike and flower scales; there was considerably less in the internodes of the stem than in the nodes.

When manganese was added (as slag) the amount of manganese in all the plant organs and their parts increased. In addition, the absolute weight of the grain and its yield also increased. The yield of wheat increased by 2.5 centners per hectare.

In 1955, in greenhouse experiments with corn (planted in 40 kg vessels) grown on meadow-chernozem-podzolic soil taken from near northern Zhuliana in the vicinity of Kiev, we experimented with the different forms and methods of adding manganese and micronutrients; we studied the absorption, translocation, and localization of manganese in individual polar parts of plant organs. The results of these investigations are given in Table 5.

Comparing the data given in Table 5 with that in Table 3, one can see that compared with winter wheat, corn is inherently less selective in the absorption and fixation of manganese. Insignificant amounts of manganese in the seeds, and a somewhat greater accumulation in the leaves and stems is evidence that manganese fertilizers should be used during the sowing of corn. This is also confirmed by a more intensive entrance of manganese into the plants when weakly soluble (manganese slags) and very soluble (manganese sulfate) forms of manganese compounds were introduced into the soil. Triple doses of manganese compounds favored a sharp increase in the manganese content of many plant organs. Furthermore, the amount of manganese in the stem and leaf components was considerably less in the base than in the oppositely located apical end which was the younger, growing, and more viable part of these organs. The increase in manganese content of corn, as well as winter wheat, progressed upward from the base and was gradual.

In our experiments the addition of different forms of manganese fertilizers to the soil favored the increase in total weight, and the total weight

TABLE 4

The Yield of Grain in Winter Wheat and Its Component Elements at Various Conditions of Plant Nutrition

System of nutrition	Length in cm		Number of grain per spike	Weight of 1000 grains in g	Yield of grain in centners/hectare	Increase of yield in centners/hectare
	plant	spike				
Organic-mineral	112.6	7.9	30.0	32.8	26.4	—
Organic-mineral + manganese slag	134.0	9.3	34.6	38.4	28.9	+2.5

TABLE 5

Manganese Content of Corn Plants (in % dry weight)

Experimental design with an organic-mineral supply	Stems	Leaves	Core of cob	Grain	Husks of cob	Suckers
Ordinary granulated superphosphate	5.17×10^{-4}	3.37×10^{-3}	5.81×10^{-4}	4.3×10^{-4}	6.83×10^{-4}	1.24×10^{-3}
Manganese enriched superphosphate	5.25×10^{-4}	3.53×10^{-3}	7.42×10^{-4}	8.9×10^{-4}	7.61×10^{-4}	1.38×10^{-3}
Ordinary powdered superphosphate	4.80×10^{-4}	3.16×10^{-3}	3.47×10^{-4}	6.30×10^{-4}	6.88×10^{-4}	1.33×10^{-3}
The same as above mixed with manganese slag	4.88×10^{-4}	4.49×10^{-3}	5.64×10^{-4}	1.29×10^{-3}	7.09×10^{-4}	1.37×10^{-3}
The same as above mixed with manganese sulfate	5.80×10^{-4}	2.67×10^{-3}	3.14×10^{-4}	9.40×10^{-4}	7.67×10^{-4}	1.44×10^{-3}
The same as above mixed with 3 doses of manganese sulfate	7.45×10^{-4}	4.61×10^{-3}	5.90×10^{-4}	1.20×10^{-3}	1.25×10^{-3}	1.29×10^{-3}
The same as above mixed with 3 doses of manganese sulfate + potassium iodide	8.74×10^{-4}	4.63×10^{-3}	4.23×10^{-4}	1.32×10^{-3}	9.11×10^{-4}	1.83×10^{-3}
The same as above, seeds treated with a 5% solution of manganese sulfate	3.00×10^{-4}	2.42×10^{-3}	2.62×10^{-4}	9.60×10^{-4}	4.31×10^{-4}	1.33×10^{-3}
The same as above, seeds treated with a 5% solution of manganese sulfate + 0.005% solution of anevrin	4.48×10^{-4}	3.12×10^{-3}	2.73×10^{-4}	1.11×10^{-3}	5.02×10^{-4}	1.50×10^{-3}
The same as above, seeds treated with a 0.005% solution anevrin	3.71×10^{-4}	9.40×10^{-3}	2.60×10^{-4}	6.80×10^{-4}	4.54×10^{-4}	1.52×10^{-3}
The same as above, seeds treated with a 0.001% solution 2,4-D	3.92×10^{-4}	9.60×10^{-3}	1.53×10^{-4}	6.20×10^{-4}	6.76×10^{-4}	1.23×10^{-3}
The same as above, seeds treated with 0.001% solution 2,4-D + 0.05% solution manganese sulfate	2.62×10^{-4}	9.02×10^{-3}	3.42×10^{-4}	7.60×10^{-4}	5.74×10^{-4}	1.34×10^{-3}

of 1000 grains, as well as an increase in protein and starch content of the corn grain. When manganese enriched granulated superphosphate was added, the weight of 1000 corn grain increased by 19.5 g as compared with the ordinary granulated superphosphate. When manganese sulfate was added it increased only by 7.7 g. When the dosage of manganese sulfate was increased, the weight of the corn grain decreased by 34.7 g per vessel. The addition of potassium iodide decreased the yield somewhat, but it did not completely remove the toxic effect of high doses of manganese sulfate; consequently in meadow-chernozem-podzolic soils this procedure was not effective.

Presowing treatment of corn seeds with a 0.05% solution of manganese sulfate, even though it appeared to have a favorable effect on growth and the increase in weight of the corn grains, however, did not help the plants reach the same level of improvement that was obtained by adding manganese fertilizers to the soil. Presowing treatment of corn seeds with solutions of physiologically active substances (anevrin and 2, 4-D) as well as a combination of this with seed treatment consisting of a solution of manganese sulfate also proved ineffective.

SUMMARY

The distribution of manganese in different parts of organs of winter wheat and maize plants has been found to be different. The largest amount of manganese was detected in the young sixth internode of the winter wheat stem; the amount of manganese increases from the lower to the higher internodes.

Smaller amounts of manganese were detected in the stem and leaf bases of maize than in the younger apical parts of these organs.

The largest amount of manganese in winter wheat and maize plants was found in the leaves. Application of manganese fertilizers led to an increase of the manganese content in all the plant organs.

Granulated manganese-containing superphosphate and manganese slag enhanced the crop yield and improved the quality of the grain of wheat and maize.

Compared to the yield of the control plants, which was 26.4 centners per hectare, that of winter wheat grown on meadow black soil in the vicinity of Kiev was found to be 2.5 c/h higher; the weight of the maize grain obtained in greenhouse experiments on the same soil was found to be 18-19 grams higher per vessel, the control yield being 99.2 grams.

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THE EFFECT OF K, Cl AND SO_4 IONS ON THE TRANSPIRATION RATE OF CROP PLANTS

I. E. Rogalev

Scientific Institute of Fertilizers, Insecticides and Fungicides NIUIF, Moscow

The water exchange of plants and the degree of water saturation of the plant tissues is related directly not only to the external environmental conditions during growth, but also to internal factors which determine the degree of the water holding capacity of the hydrophilic biocolloids of the protoplasm.

The supply of essential nutrient elements plays a substantial role in regulating the water exchange of plants. Mineral nutrients which participate in the formation of the structural elements of proteins and regulate living processes are also important in regulating the water relations of a plant.

The late investigations by Alekseev and Gusev [1-4] established the fact that nitrates and phosphates have a great effect on the water relations of wheat and the state of water in the tissues; they concluded that it is possible to direct the water relations of a plant by means of regulating the essential mineral nutrients.

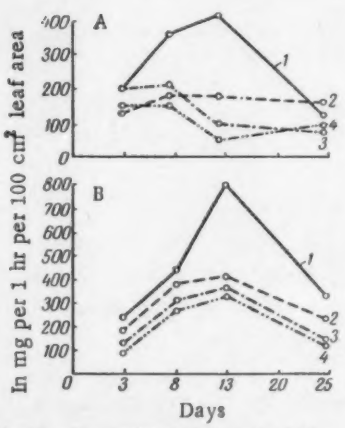

Fig. 1. Transpiration in corn (A), and in winter wheat (B).
1) Control (without potassium); 2) 0.1 N K from Knop's solution; 3) 1.0 K 4) 5.0 K.

The attention of investigators had turned to the significance of potassium in the water exchange of plants considerably earlier because of phenological observations on the appearance of external symptoms of potassium deficiency [5-9] which could be generally summarized as follows: a) decrease in leaf turgor, which was especially rapid in warm and dry weather; b) a change in the color of the leaves in the last stages of deficiency, this began in the lower leaves where the margins gradually turned yellow or became brown; c) curling of the leaf margins and their gradual death.

After the lower leaves had been affected the leaves of the remaining layers changed in the same way, consequently the growth of the plants was inhibited and as a result a minimum yield was obtained.

The earlier experiments pertaining to a study of the effect of potassium on the water exchange of plants under specific conditions can be divided into two groups:

1. Laboratory and greenhouse experiments concerned with the antagonistic effect of potassium and calcium on the water relations of plants in which it had been observed that potassium had a favorable effect on the water balance of the plants; at the same time potassium was conducive to a more economical use of water in comparison with plants which were kept in solutions of calcium salts [10-13]. This is in complete agreement with data from cellular observations concerning the effect of potassium on the hydration of protoplasm and the effect of calcium on its dehydration [14, 15].

2. Greenhouse experiments and field observations concerning the effect of potassium sales on the rate of transpiration made at various soil climatic conditions and with different forms of potassium salts; these gave

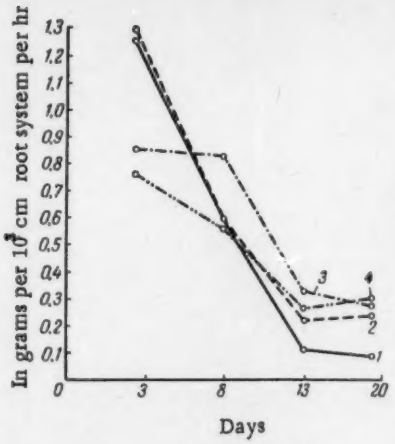

Fig. 2. Water absorption by excised corn roots at various levels of potassium in the nutrient medium.
1) Control without potassium; 2) 0.1 N K in Knop's solution; 3) 1.0 K; 4) 5.0 K.

contradictory results. In one group of experiments potassium fertilizers were observed to have a favorable effect on the decrease of water utilization by plants [16-18]. In another group of experiments data were obtained which indicate that the rate of transpiration was greater in the plants with an adequate potassium supply [19-21]. However in these investigations no attention was given to the effect of the anions which were components of the potassium salts; they could possibly have a very important effect on the water exchange of plants.

In our greenhouse experiments with potassium salts during 1953-1955 using water and soil cultures at the experimental base of the Scientific Institute of Fertilizers and Insecticides and Fungicides at the Dolgoprudnaia Experiment Station (DAOS) - we were interested in the effect of the chlorine and sulfur anions, which are components of the potassium salts, on the rate of transpiration.

METHODS

The following plants were used in the experiments: sunflower, variety early Saratovskii; red clover; winter wheat, hybrid 599; corn, varieties Uspekh and Bezenchukskii; and barley, variety Vinner.

The present paper also contains data from experiments by the author performed earlier at the Moscow Botanical Garden concerning the effect of potassium on the rate of transpiration in corn, variety Bezechukskii, and winter wheat 02411 in the early stage of vegetative growth (from 12 to 37 days growth).

The experimental data presented below were obtained in experiments which were performed as follows.

A) Water culture with Knop's nutrient solution containing an amount of sodium equivalent to the exchangeable potassium. I, 0.01 K + Na; II, 0.1 K + 0.9 Na; III 0.1 K; and IV, 5.0 K.

B) Water culture with Hellriegel's [1] nutrient solution with potassium chloride in one variant and an equivalent amount of potassium sulfate in the second variant. I, KCl; II, K_2SO_4 and III, KCl $\frac{1}{2}$ + K_2SO_4 $\frac{1}{2}$. Phosphorus was added as NaH_2PO_4 and magnesium as $Mg(NO_3)_2$.

The microelements boron, manganese and iron were added to the nutrient solution.

C) Soil culture on heavy clay podzolic soil which was first neutralized by the addition of calcium carbonate at the rate of 0.83 g per 1 kg soil. In this experiment in which the supply of nitrogen (170 mg) and phosphorus (20 mg P_2O_5 per 1 kg soil) was adequate, we investigated the effect of potassium chloride and potassium sulfate on the rate of transpiration

In the experiment with sunflower, corn, and clover the potassium content of the soil before adding fertilizer was equal to 49 mg per 1 kg soil. Eighty-eight mg of potassium per 1 kg of soil were added with the potassium fertilizer.

The transpiration rate of plants growing in water cultures was measured by the weight method using an apparatus constructed on the same principle as that used by Vesk. Entire plants were placed into the assembled apparatus and then placed into a glass chamber in which the light, moisture, air and temperature conditions were kept constant.

The transpiration rate measurements of plants grown in soil cultures were made on leaves which had been excised in liquid paraffin and then also placed into a chamber at constant conditions.

The transpiration rate measurements were continued for 3 hours. The results of the measurements concerning the amount of water transpired in experiment A measured for 1 hour according to Dalton's formula 3, 8, 13, 20 and 25 days after the beginning of the experiment were calculated on the basis of one unit of leaf area; in

TABLE 1

The Effect of Chlorine and Sulfur Anions on the Transpiration Rate of Winter Wheat

Experimental variants	Transpiration		% water content on basis of dry weight
	in mg per 1 g fresh weight of leaves	in g per 1 g constant dry weight	
KCl	326	2.23	582
K_2SO_4	495	4.00	710
KCl ½ + K_2SO_4 ½	388	3.02	664

TABLE 2

Transpiration Rate of Experimental Plants in the Vegetative Stage

Experimental variants	Corn		Sunflower		Clover	
	on fresh weight basis, in mg/g	on constant dry weight basis, in g/g	on fresh weight basis, in mg/g	on constant dry weight basis, in g/g	on fresh weight basis, in mg/g	on constant dry weight basis, in g/g
NP	196	2.04	221	–	181	1.24
NPKCl	156	1.58	205	2.74	152	1.08
NPK_2SO_5	176	1.91	253	3.98	222	1.60
NPKCl ½ + K_2SO_4 ½	–	–	208	3.24	193	1.39

TABLE 3

Transpiration Rate of Experimental Plants During the Booting Stage and Maturation of the Seed

Experimental variants	Booting stage		Seed maturation	
	on fresh weight basis, in mg/ g	on constant dry weight basis, in g/g	on fresh weight basis, in mg/g	on constant dry weight basis, in g/g
NP	207	1.34	207	0.93
NPKCl	171	1.19	184	0.63
NPK_2SO_4	183	1.36	280	1.10
NPKCl ½ + K_2SO_4 ½	159	1.15	258	1.05

experiments B and C the amount of water transpired was calculated on the basis of 1 g fresh weight and 1 g constant dry weight of the transpiring organ. The total water content was obtained after drying the experimental samples at 105°.

In addition, the activity of the root system of corn growing in water culture in the absorption of water was calculated by measuring the rate of exudation by the conventional means after the aerial part of the plant was excised. In this way the rate of water absorption by the root system was determined after certin time intervals

(3, 8, 13 and 20 days). At the end of the experiment the volume of the root system was determined [22]. The final calculation of the amount of water absorbed for each unit volume of root system made it possible to evaluate the activity of the root system in the process of water absorption and its relative permeability.

Results of Experiments on Potassium Nutrition and Transpiration

The results of the periodic determinations of the transpiration rate of young corn and wheat plants over a period of 3 to 25 days in relation to the effect of the presence or absence of potassium in the nutrient medium on plants grown in the corresponding medium are represented graphically in Fig. 1.

From Fig. 1 one can see that the greatest rate of water loss by the plants occurred when potassium was completely absent from the nutrient medium. Furthermore, the progressing rate of water loss reached its maximum by the 13th day of the experiment; this coincided with the initiation of the branching stage of winter wheat and the beginning of rapid growth in corn.

It can be assumed that this is associated with the migration of potassium from the leaves into the areas of shoot initiation in winter wheat and to the growing points in corn since potassium is localized to a considerable degree in the meristematic tissue and young growing parts of plants [23].

The loss of potassium from the transpiring organs resulted in a decrease of their water holding capacity, consequently the water which entered the root system because of the osmotic pressure of the subterranean organs rapidly evaporated. The presence of a small amount of potassium in the nutrient medium (0.1 N K from Knop's) in any stage of plant growth significantly decreased the rate of transpiration; this is also evident from Fig. 1. An increase in the potassium dosage (5.0 K) at the same nitrogen and phosphorus dosage did not induce a noticeable change in the rate of water loss when compared with plants on a normal potassium supply (1.0 K).

In order to be sure of the relationship between the transpiration rate of the aerial organs and the water-holding capacity of their constituents it was necessary to establish a parallel relationship between the degree to which the root system was permeable to water when it was isolated from the effects of the osmotic pressure of the sap in the aerial organs. The results of these determinations are given below.

Permeability of Roots to Water

The increased loss of water by the plants during a critical potassium defficiency in the nutrient medium could, to a considerable degree, be associated with the activity of the root system and its permeability to water (in investigations with entire plants).

The results of periodic determinations on the rate of water absorption by the root systems of corn isolated from the aerial part of the plant are given in Fig. 2.

Figure 2 shows that when potassium was absent from the nutrient medium, and also when it was almost lacking (0.1 N), during the first three days after the beginning of the experiment the rate of water absorption by the root systems was greater and considerably exceeded the level of absorption by plants well supplied with potassium. At higher doses of potassium the permeability of the roots to water decreased somewhat in the first period, and within 20 days it was almost equal to that in plants with a normal potassium supply. The increased permeability of the roots to water when the potassium supply was inadequate, and the somewhat decreased permeability when potassium was abundant during the first period of the experiment, can be explained by the variation in the water holding capacity of the protoplasm in root cells through which the water is absorbed and translocated; it decreased during a potassium deficiency and increased when potassium was present.

There is no doubt that either high or low protoplasmic permeability to water is inevitably due to the organization of the protoplasm and the stage of water in it [24].

During an extreme potassium deficiency (13-20 days) the water absorbing activity of the roots decreased considerably in comparison with that in plants of the potassium series.

The data above concerning the effect of potassium on the rate of water absorption and its evaporation shows that when the potassium deficiency of the nutrient medium was critical first there was a change induced in the water holding capacity of the root cells, and then (within 8-13 days), the leaves become depleated of potassium, and finally there was a migration of potassium to the growing points. This induced an increased rate

of water loss, and as a result a decrease in the water holding capacity of the aerial plant organs [25, 26].

The Effect of Chlorine and Sulfur on Transpiration

The experiment described above concerning the effect of potassium on transpiration was performed using Knop's nutrient medium containing chlorine and sulfur in which the specific effect of these elements on transpiration was constant.

The results of the experiment performed with winter wheat according to design B in which the effect of the chlorine and sulfur anions on the transpiration rate of 13-day-old plants in the corresponding nutrient solutions was determined are given in Table 1.

The highest transpiration rate was observed in plants grown on a nutrient medium in which potassium was used in the form of potassium sulfate. When the presence of chlorine and sulfur anions in the nutrient medium was in the ratio of 0.5 N: 0.5 N, as used in the experiment, the transpiration rate was lower than when only potassium chloride was used. Therefore, because of the sulfate nutrition the water content of the plants in our experiment was higher than in plants which had grown in a nutrient solution with the chloride ion. The increased water content of plant samples following the diffusionof potassium sulfate into them was established by Shcherbakov [33].

The varying effect of chloride and sulfate salts of potassium on the evaporation of water was also observed in our experiments with various plants from soil cultures. Table 2 contains the results of transpiration rate determinations for corn, sunflower, and clover plants during the vegetative stage.

The data presented in Table 2 shows that the presence of chlorine in the nutrient medium, introduced as potassium chloride, brought about a decrease in the transpiration rate of plants in soil cultures. On the other hand the presence of sulfur in the nutrient medium of the substrata in an easily accessible compound favored a higher transpiration rate of these plants.

Mixtures of potassium chloride and potassium sulfate brought about somewhat of a decrease in the evaporation rate as compared with plants from the variants with only potassium sulfate, and a noticeable increase in comparison with plants from the variant with only potassium chloride.

The progressive effect of chloride on the retardation of the transpiration process of barley plants grown in soil cultures, from the booting stage to the stage of seed maturation, can be seen in Tabel 3.

Hence, the results of determinations on the transpiration rate of the indicated plants grown in water and soil cultures very clearly show the retarding effect of chlorine on the transpiration process. In the meantime sulfur had a stimulating effect on the rate of transpiration in sunflower and clover in soil cultures.

Warne [27] presents some data concerning the retarding effect of chlorine on the transpiration rate of buckwheat in experiments performed in the greenhouse during the winter.

DISCUSSION AND EXPERIMENTAL RESULTS

The data presented here obtained from experiments performed at conditions of normal doses of chlorine or sulfur in the nutrient medium agree completely with the results of experiments which Genkel'et al. [28] obtained during a study of the physiological processes typical of halophytes, cotton, and other plants under conditions of excessive quantities of chloride and sulfate salts. The decreased transpiration rate observed in these experiments during an excess of chloride was accompanied by a retardation of the other physiological processes — photosynthesis, respiration, enzyme activity of some of the enzymes, and a decrease in chlorphyll content.

Similar changes in the energy of assimilation, chlorophyll content, and also an increase in the process of starch digestion in potatoes due to the influence of the chlorine ion were also observed in experiments in which chlorine-containing salts were used in the fertilizer [29-31].

Hence, the inhibition of transpiration due to the effect of the chlorine anion is accompanied by a slowing down of other physiological functions as well. The mixtures of potassium chloride and potassium sulfate which we used in our experiments had a favorable effect on the rate of transpiration; they induced an increase in the rate as compared with plants supplied only with potassium chloride.

The diverse effect of chlorine and sulfur on the transpiration rate as well as other physiological functions of the plant is undoubtedly associated with their different role in the metabolic processes. The decrease in the rate of protoplasmic streaming in Elodea due to the influence of cadmium chloride and the increase in the rate of protoplasmic streaming when the sulfhydryl group is present in the medium which was observed in Smirnov's experiment [32] indicates positively the essential role of the sulfhydryl group on "delicate chemical processes which are basic to the activity of protoplasm", as well as in intracellular metabolism which contribute to activating the cellular physiological functions.

In regard to the question concerning the effect of potassium on the transpiration rate of plants, it should be noted that when potassium was lacking or when it was replaced by sodium in the water cultures, the rhythm of water exchange in the transpiration process was sharply disrupted, as we showed above. The period of highest transpiration was usually followed by the appearance of external symptoms of potassium deficiency, and the leaves which had the highest water exchange gradually died. But in this very experiment the addition of a comparatively small dose of potassium (0.1 N Knop's) at any stage of plant growth or development induced a decrease in the water exchange of the plants as compared with plants from the variant without potassium (see Fig.1).

In the soil cultures without potassium fertilizer where the soil content of potassium was 49, or in another experiment 68, mg per 1 kg soil there was no increased transpiration rate observed in comparison with plants which were grown in soil fertilized with potassium sulfate or a mixture of potassium chloride and potassium sulfate. Apparently the mobilization of potassium as the result of the activity of the root system and the absorption of potassium from the soil was provided for by the normal physical-chemical conditions in the plant cells. Under these conditions the disruption in the water retaining properties of the plant organs apparently did not occur, and consequently did not bring about any noticeable disruption in the exchange of water at the stages of plant growth and development indicated. However, the rate of transpiration in the plants from the variant " without potassium fertilizer" was, as a rule, higher than it was in the plants which had grown in soil enriched with potassium chloride (see Tables 2 and 3).

There is no doubt that the chlorine ion has a retarding effect on the transpiration process.

SUMMARY

1. During a critical potassium deficiency the transpiration rate increased significantly as a result of the decrease in the water retaining properties of the transpiring organs, and reached a maximum at the beginning of the booting stage in winter wheat and in the early stage of rapid growth in corn.

2. The presence of chlorine in the nutrient medium added as potassium chloride, retarded the transpiration process considerably in plants from different systematic groups.

3. The presence of the SO_4 anion in the nutrient solution induced an increase in the transpiration rate of sunflower and clover.

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PARTICIPATION OF GROWTH SUBSTANCES OF THE AUXIN TYPE IN FORMATION OF TUMORS ON POTATO PLANTS

T. A. Reingard and S. I. Pashkar'

All-Union Scientific-Research Station of Potato Tumors, Shernovitsy

The nature of vegetable tumor formations has lately attracted wide attention.

Among the factors causing tumor formation in plants, a prominent place is given to growth substances. Numerous studies in this direction were conducted chiefly on tumors of bacterial origin, and are summarized in a number of surveys [1-4 and others]. Even though, according to these studies, the formation of vegetable tumors by pathogenic agents cannot be described as a simple reaction of the plant to the formation of growth substances by the stimulant, and the primary cause of the vigorous rise of nondifferentiated growth still remains unclear, nevertheless it may be considered established that in the formation of bacterial tumors in plants, the growth substances participate at some stage of their formation.

Regarding participation of growth substances in formation of tumorous swellings in potatoes when infected by the fungus Synchytrium endobioticum (Schilb.)Perc., up to the present time there is only a single indication, by Sukhorukov [5]. The author did not detect the presence of growth substances in alcohol extracts of tumors when they were tested for leaf epinasty of young tomatoes. However, the presence of indole in old growths was established qualitatively, as well as traces of this substance in young growths. From the results obtained, the author concluded that there was "an absence of growth stimulators of the auxin type in the growths."

The stimulator of potato tumors — the fungus Synchytrium endobioticum — occurs as a typical obligate parasite, usually infecting young growing parts of all potato organs except the roots. Among the varieties of cultivated potatoes (Solanum tuberosum), there are types which are susceptible as well as resistant to the tumor-stimulator. The fungus zoospores embedded in the tissues of the susceptible host-plant cause a profound disturbance of metabolism which is reflected in vigorous cell fission accompanied by tumors. In tissues of a resistant variety, the embedded fungal zoospores do not give rise to formation of tumor growth.

A number of studies have been devoted to the investigation of potato growth substances, chiefly during the last decade [6-14]. In these studies the tubers were the chief subject of investigation. There are indications of the presence of stimulators in potato tubers [6-11] as well as growth inhibitors, the nature of which is still remote and unclear [7, 12-14].

The purpose of this investigation was to establish the extent to which growth substances participate in the process of vigorously expanded growth of susceptible varieties, and to clarify whether the susceptible and resistant potato varieties differ in their content of growth substances. It was equally of interest to clarify whether there are qualitative differences between growth-activating substances of healthy and tumorous potato tissues.

METHODS

As material for the investigation we used tumerous growths, tubers, leaves, as well as sprouts of resistant and susceptible potato varieties.

In order to obtain extracts of growth substances, the fresh vegetable matter was extracted by ether or alcohol. The finely cut weighed portion was wetted with ether or alcohol in the proportion of 3 ml solvent per g

TABLE 1

Comparative Effect of Ether and Alcohol Extracts from Tumorous Potatoes on Growth of Pieces of Oat Coleoptiles (in mm per 10 pieces of coleoptiles)*

Variant	Fraction	
	Free	Bound
Ether extract	15.92	2.65
Alcohol extract	0.14	0,0
Water	4.11	

* In this and subsequent tables, the data are the average of triplicates. The difference between replicates did not exceed 0.8 mm.

TABLE 2

Action of Alcohol Extracts from Potato Sprouts on Growth of Pieces of Oat Coleoptiles (in mm per 10 pieces)

Variety	Fraction	
	Free	Bound
Lorkh	5.14	8.77
Vol'tman	8.54	9.28
Grentsmark	9.73	13.89
Karnea	8.18	7.58
Water	9.46	

TABLE 3

Comparative Effect of Extracts from Tumors and Normal Tissue of Infected Organs on the Growth of Pieces of Oat Coleoptiles (in mm per 10 pieces of coleoptiles)

Test variant	Fraction	
	Free	Bound
Infected sprouts		
Tissue of tumorous growths	10.31	7.01
Normal tissue of infected sprouts	8.35	6.44
Infected tubers		
Tissue of tumorous growths	9.55	6.71
Normal tissue of infected tubers (in the eye zone)	4.44	5.97
Water	7.14	

of vegetable matter and was steeped at room temperature. The extract obtained after a 4-hour steeping, during which the solvent was changed twice, comprised the fraction of "free" growth substance. The remaining matter was again flooded with ether or alcohol and was steeped for 18 hours. This extract comprised the fraction of "bound" growth material. Water was added to the ether and alcohol extracts in the proportion of 3 ml water per g of vegetable matter; the ether was evaporated, while the alcohol was driven off under reduced pressure. The aqueous solutions were then used for analysis.

In studying the qualitative nature of "free" and "bound" growth substances of healthy and tumorous tissues, the extracts were subjected to treatment by peroxide and acid, according to the method described by Funke and Söding [7], Meyer [12], and Baumeister [13]. To 1 ml of the extract 2 drops of a 5% solution of hydrochloric acid were added, and the mixture immediately evaporated to dryness on a boiling water bath, after which the dry residue was dissolved in 1 ml of water and the mixture again evaporated. The latter operation was repeated twice. The dry residue was again dissolved in 1 ml of water and the solution used for analysis. When treated by peroxide, the same operations were repeated, the only difference being that the first evaporation was conducted 20 minutes after addition of perhydrole. As a control the extract was tested, thrice evaporated, but without addition of peroxide or acid.

The analysis of treated and untreated extracts was conducted by two methods: a) growth of pieces of oat coleoptiles and b) paper chromatography.

a) Measurement of increment in pieces of oat coleoptiles. Ten pieces of oat coleoptiles of about 3 mm were placed in vessels with the extract being tested. Two pieces from each coleoptile were cut off 2 mm from the top. The initial length of the coleoptile pieces was measured by a telescopic lens using a screw ocular-micrometer. After 24 hours the growth of coleoptile pieces was measured. In the control vessels the growth of coleoptile pieces in pure water was measured.

In order to test the capacity of extracts to inhibit the action of heteroauxin solutions, the growth of coleoptile pieces was measured in a mixture of equal volumes of 0.001% heteroauxin solutions and the extract being tested. In these cases the control consisted of a mixture of equal volumes of 0.001% heteroauxin solution and water.

b) Paper chromatography (by a modified method described by Bitancort [15]). On strips of Leningrad rapid chromatography paper, measuring 21 x 27 cm, at a distance of 6 cm from the upper edge and 2.5 cm from

Test variant	Growth*
Tissue of leaf tumerous growths	17.02
Normal tissue of infected leaves	12.5
Water	10.1

* The effect of the entire fraction of growth substances on the growth of the coleoptiles was determined.

TABLE 4

Localization of Growth Substances in Tumors (by growth of 10 pieces of oat coleoptiles in mm)

Growth zone	Fraction	
	Free	Bound
Peripheral*	11.58	9.27
Internal*	7.22	6.98
Water	8.92	
Peripheral**	29.16	25.28
Internal**	97.46	16.66
Heteroauxin 0.001% + water (1 : 1)	18.71	

* 1:1 mixture of initial extract + water

** 1:1 mixture of initial extract of 0.001% heteroauxin solution.

from one another, 8 spots of the test extract were applied and 1 spot of the known – 0.01% of heteroauxin solution and 1 spot of a mixture of the known and the test extract. The spots were applied by a micropipette, each 6 times (of 0.01 ml, altogether 0.06 ml) with subsequent drying, after which the chromatogram was folded into a tube and attached by the upper end in a flat bowl with distilled water (ascending chromatogram). In the bell an atmosphere saturated with vapors of acetic acid and water was created. When the solvent front ascended about 15 cm (usually after 1.5 hours), the chromatogram was removed, dried, examined under ultraviolet (UV) rays, and then sprayed with Salkov reagent (see Bitancort[15], or with Bioarkin reagent.* The pink or pink-lilac developed spots were outlined by a red pencil. For preservation of the chromatograms obtained, they were rinsed in water for 30-40 minutes in order to eliminate excess sulfuric acid, and dried.

RESULTS OF TESTS

1. Choice of solvent. Growth substances are most frequently extracted from vegetable tissues by ether or alcohol.

In an especially conducted experiment we established that, in extracting growth substances from tumors and potato sprouts, it is better to use ether because alcohol extracts, as a rule, in our experiments did not produce any growth in pieces of oat coleoptiles (Tables 1 and 2).

It is doubtful whether alcohol extracts growth-promoting substances from potato tissues. Rather it can be assumed that, together with growth substances, alcohol also extracts inhibitors which mask the stimulatory activity. The latter hypothesis is substantiated by chromatographic data on ether and alcohol extracts of sprouts and tumorous growths. In both cases, in developing the chromatograms of the alcohol as well as the ether extracts, there appear clearly expressed spots with an average R_f of 0.7, close to the R_f of the heteroauxin known (in pure form, on the average 0.82; in a mixture with the extract, on the average 0.71).

It is of interest that when the chromatrograms are examined under ultraviolet prior to spraying by the developer, the alcohol extracts always display a blue-violet fluorescence at or alongside of spots of later pink coloration, while the ether extracts display almost no fluorescence under ultraviolet. Hence, there may be an inhibiting role of the fluorescence factor under ultraviolet rays. In the light of the data cited, it is understandable why in Sukhorukov's [5] experiments with alcohol extracts from tumors, no epinasty in tomatoes was observed. In the subsequent experiments we used only ether extracts.

2. Comparative content of growth substances in a tumorous growth and normal tissues of infected organs. Results of all experiments conducted by us indicate that more growth-stimulating substances are concentrated in

* This reagent was used by A. N. Boiarkin for colorimetric and histochemical determination of B-indoleacetic acid. The A. N. Boiarkin reagent consists of a mixture of an aqueous solution of ferric alum (0.1%) and concentrated sulfuric acid (3 : 1). We used these components in a 1 : 1 mixture.

TABLE 5

Comparative Effect of Extracts from Sprouts of Resistant and Tumor-susceptible Potato Varieties for Growth of Pieces of Oat Coleoptiles (in mm per 10 coleoptile pieces)*

Potato varieties	Fraction		Potato varieties	Fraction	
	Free	Bound		Free	Bound
Resistant			Susceptible		
Kornea	3.42	6.62	Krasnodarets	4.65	5.57
Oktiabrenok	1.94	4.06	Selanets 36	4.29	4.89
Ostbote	5.66	6.15	Sovetskii	3.41	6.89
Grentsmark	3.32	4.39	Epron	5.28	5.24
Mazhestik	2.86	4.39	Stakhanovskii	3.28	4.77
			Water	1.80	

* Original extracts diluted with water 1:1.

TABLE 6

The Action of Extracts from Healthy (Uninfected) Sprouts and Tumors of Vol'tman and Vale Varieties Treated by Acid and Peroxide on Growth of Pieces of Oat Coleoptiles (in mm per 10 coleoptile pieces)

Test variant	Fraction	Without treatment	Control heated	Treated by	
				Acid	Peroxide
Vol'tman variety					
Sprouts	Free	5.65	6.77	6.46	6.20
	Bound	5.68	4.16	6.70	6.47
Growths	Free	7.61	4.84	5.08	3.02
	Bound	4.29	5.73	5.03	1.77
Water		4.74			
Vale variety					
Sprouts	Free	14.56	17.53	13.46	13.59
	Bound	15.95	13.72	12.85	15.38
Growths	Free	13.6	12.49	9.05	8.35
	Bound	12.85	12.00	9.92	9.65
Water		7.42			

the tumors than in the healthy portion of the organ on which the growth was formed (Table 3).

The effect of extracts from green leaf-growths and normal tissue of infected potato leaves on growth of pieces of oat coleoptiles (mm per 10 pieces of coleoptiles) are given in the Chart on page 514.

Thus, the formation of tumorous growth is accompanied by the formation of considerable quantities of growth substances in it.

It should be noted that if completely healthy potato sprout or leaf tissue, uninfected by fungus, is compared with a tumorous growth formed on another plant, the content of growth substances in the latter is frequently lower. This evidently depends on the age of the organ or growth when taken for analysis.

The data of chromatographic analysis (the spot intensity) confirm the results of biological tests as given in Table 3. However, in testing extracts from leaves (experiments were conducted in August and September), not

TABLE 7

Effect of Extracts from the Healthy Tissue of Infected Tubers of Vol'tman Variety and Tumors, Treated with Acid and Peroxide, for Growth of Oat Coleoptile Pieces (total fraction) (in mm per 10 coleoptile pieces)

Test variant	Control		Treated by	
	Without	Heated	Acid	Peroxide
Tuber (eye zone)	2.83	3.78	3.38	4.60
Tumor	4.78	4.53	6.40	2.66
Water		3.89		

in a single case did we observe a concordance of biological test data with the chemical qualitative test. While the ether extracts from leaves always stimulated growth of oat coleoptiles, not once in our experiments did they produce any color with A. N. Boiarkin reagent which, evidently, indicates the presence of another type of growth stimulating substance in leaves.

3. Localization of growth substances in tumors. Potato tumors are not homogeneous formations, but are composed mainly of two zones, an outer meristematic one with numerous dividing cells and an inner one which includes conducting tracts and parenchymal cells. Therefore we were interested in the question of growth substance distribution in the tumor zones.

As the data in Table 4 show, the growth substances of tumors, as was expected, are chiefly concentrated in their external corrugated portion. The data as to coleoptile growth are confirmed by paper chromatography of extracts from both zones of the tumor. While the extracts from the outer zone when developed by Boiarkin or Salkov reagent produce a distinctly pink spot (average R_f 0.7), the extracts from the inner zone produce a very light color, even after heating.

4. Growth substances of healthy sprouts of resistant and tumor-susceptible potato varieties. To clarify possible differences in the content of growth substances between the susceptible and tumor-resistant potato varieties, we analyzed sprouts of 5 susceptible and 5 tumor-resistant varieties (Table 5).

There are no uniform differences in absolute content of growth substances between susceptible and tumor-resistant potato varieties. Even the apparent tendency of a higher content of the bound fraction, by comparison with the free one, in the sprouts of resistant varieties, was not always confirmed in subsequent experiments. Also no differences in distribution were found by paper chromatography between growth substances in sprouts of susceptible and resistant potato varieties.

It is known that synthetic preparations of growth substances, when applied to vegetable tissues in a mixture with lanolin paste, can cause expansion of tissues with formation of swellings.

Based on the possibility that a fungus — a tumor stimulant — in penetrating tissues of potato plants liberates growth-activating substances causing hypertrophy of tissue only in susceptible varieties, it can be assumed that the action of synthetic growth substances can produce a different reaction in susceptible and resistant potato varieties. In order to verify such a hypothesis, we tested the action of lanolin paste with heteroauxin (3% and 1%) by application to sprouts (3-4 cm long) of susceptible varieties: Vale, Ella, Ranniaia roza, Stakhanovskii, Vol'tman, Al'ma, Lorkh, Rizafoliia; and resistant varieties: Grentsmark, Karnea, Friumelle, Oktiabrenok, Parnassiia, Ostbote, Mazhestik, Iubel'. After application, the resistant as well as susceptible varieties began to form swellings in 2 days; although in some cases the tendency to form swellings was more evident in the susceptible varieties.

The possibility is not excluded that at lower heteroauxin concentrations or even in testing other substances which may prove to be more specific in this regard, differences can be established in reactions of susceptible and resistant potato varieties.

5. Differences between growth substances of healthy and tumor tissues of potatoes. In order to clarify the nature of growth stimulating substances in healthy and tumor potato tissues, extracts from sprouts, tubers and potato tumors were treated with acid and peroxide (Tables 6, 7).

The data shown in Tables 6 and 7 indicate that, in contrast to healthy tissues, potato tumors exhibit a growth factor sensitive to peroxide action.

At the same time, in their reaction to acid the growth substances of healthy and tumor tissues do not manifest any regular differences.

DISCUSSION

Tests of ether extracts from potato tumors by qualitative tests for the indole ring and by the method of oat coleoptile growth consistently indicate a considerable content of auxin-type growth substances. Unfortunately, we are thus far unable to decide whether these substances are metabolic products of unhealthy host-plant tissues or are evolved by the fungus. The fungus Synchytrium endobioticum, an obligate parasite, cannot be successfully cultured. Accordingly it is extremely difficult directly to examine the products it liberates.

The extracts from tumors always consist of a mixture of substances isolated from diseased tissues of the plant-host and from the body of the fungus-stimulant. In any case, the presence of the auxin-type growth substances in tumors, evidently, indicates their participation in forming a tumorous swelling. In the light of current opinions as to development of vegetable growths [2, 3], it can be assumed that the primary stimulus to the host's cell division emanates from the fungus stimulant, while in the course of the growth which has already begun, the formation of growth substance is carried on by the plant tissue itself.

The attempt made by us to elucidate the qualitative properties of growth substances in tumorous and healthy, nonswollen potato tissues indicates the uniqueness of tumor growth substances. Thus, their greater sensitivity to effects of hydrogen peroxide was observed by comparison with the sensitivity of growth substances in tissues of healthy potato organs. The tendency to a larger content of the free fraction in the growth should be noted by comparison with the bound fraction, while a contrary proportion is frequently observed in sprouts and tubers. In this connection, the study of Bol'shakova [16] is of interest, in which it is shown that in tumorous growths principally free "bios" is contained, while in sprouts the bound always predominates. Evidently, the intensive growth in formation of a tumor requires a greater mobility of growth-stimulating substances, which concentrate at this time, evidently, in the free fraction. A similar relationship between the free and bound fractions of auxin-type growth substances is also observed in the formation of swellings on other plants and by other stimulants [17].

In this connection, the presence of differences could be expected in the relationships of free and bound fractions of susceptible and resistant potato varieties, accounting for the capacity to rapid formation of swellings by the susceptible varieties penetrated by the parasite. No clear differences were noted by us in this direction. Further studies, however, appear to us worthwhile, in which special attention should be given to the quantitative and qualitative composition of substances stimulating and inhibiting growth.

SUMMARY

Formation of tumors in potato plants is accompanied by the formation of large amounts of growth substances. Tumors contain more growth stimulating substances than do healthy tissues of the diseased organ. Growth substances in tumors are localized in the peripheral zone of intense growth.

The free fraction of growth substances of the tumor is large compared to that of the healthy shoots of potatoes.

Tumors contain larger amounts of growth substances sensitive to peroxide than do nontumorous shoot and tuber tissues.

No distinct difference was detected in the total content and in the ratio of free and bound fractions of growth substances in shoots of wart-resistant and in shoots of wart-susceptible varieties of potatoes.

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PHYSIOLOGICAL PROPERTIES OF RINGED BRANCHES OF CITRUS PLANTS

T. V. Nekrasova

K. A. Timiriazev Institute of Plant Physiology, Academy of Sciences, USSR, Moscow

Ringed bark cut-outs are widely employed both in scientific investigations in plant physiology as well as for practical purposes. Gardeners use ringing to intensify fruit bearing as well as to hasten the flowering of fast-growing but slow fruit-bearing trees. Recently ringing has been used to speed up fruit-bearing seedlings of fruit trees, including citrus cultivations [1-4]. In the experiments of Furr, Cooper, and Reece [2], 3-year-old seedlings of grapefruit and oranges flowered, and in the experiments of Ivanovskaia [3] and our own jointly with Chailakhian [4], lemon seedlings flowered in the 5th year, while ordinarily citrus seedlings under natural growing conditions enter the fruit-bearing stage in the 8th - 12th year of life [5].

The effect of ringing is frequently explained by a slowing of assimilant flow and an increase in their concentration in the ringed part of the tree. Chendler [6] points out that ringing increases the carbohydrate concentration and decreases the nitrogen content in tissues above the ringed portion. However, the physiological changes are not limited to this, since ringing introduces more profound changes of metabolism in the ringed portion of the plant. In ringing, the interaction of leaves and roots is disturbed and, as Ivanov [7] points out, this "leads to a marked intensity of the oxidation — reduction potential of leaves and to the disruption of their physiological function." Ringing exerts a substantial effect also on growth processes. In our experiments with lemon seedlings, conducted jointly with Chailakhian [4], the ringing of the stem or branches at first caused a delay in and then a total stoppage of shoot growths, and yellowing and shedding of leaves. The restoration of interconnection between leaves and roots in ringing, however, brings on a renewal of shoot growth. This was observed in our experiments when ringing was done using lanolin [8], and also in Shishkin and Murinson's experiments [9], as well as in Vinokur's [10] experiments when air roots were formed on ringed branches.

Although ringing causes a certain disturbance in the normal course of plant metabolism, nonetheless a study of metabolic characteristics in ringing is of interest, especially for clarifying conditions which aid transition of citrus seedlings to the stage of flowering and fruit bearing.

METHODS AND EXPERIMENTAL CONDITIONS

In order to study physiological characteristics of ringed shoots, we conducted special experiments in 1954, 1955, and 1956, in which 4-6-year-old Novogruzinsk lemon seedlings and 6-year-old Shvetsov Seianets orange seedlings were tested. In 3 tests all the plants were ringed simultaneously, and in one test the ringing was done at different times. In the first test the ringing was done on January 26, 1954; in the third test on June 16, 1955; in the fourth test on June 1, 1956; and in the second test on December 30, 1953, April 14, May 8, June 1, and June 25 of 1954. The ringing was done on the upper branches consisting of one or several sheets. In experiment 4 on lemon seedlings, sizable 2-year-old branches, grown after extensive trimming of the plant, were ringed. These branches were from 50 to 90 cm in length. Two similar branches were chosen on each plant, of which one was ringed; the other, not ringed, served as a control. Before ringing, all the shoots, including the controls, were pinched.

Samples were taken at various times; at 2-3 weeks and later. In the first test the samples were taken on February 15, March 11, April 8 and July 26; in the third test, on July 1, July 15, July 29, and August 17; and in the second and fourth tests the samples were taken during July and August; moreover, in the second test the samples were always taken simultaneously from all periods of ringing.

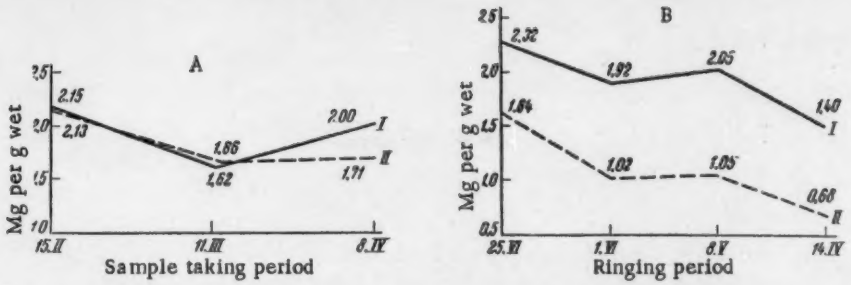

Fig. 1. Chlorophyll content in leaves of ringed and unringed lemon shoots (mg per g wet weight).
I) Control; II) ringed. A) Data of first test; B) data of second test; I) control; II) ringed.

TABLE 1

Pigment Content of Leaves of Ringed and Nonringed Lemon and Orange Shoots

Plant species	Experimental variant	Content, in mg per g wet weight			Ratio of carotene to xanthophyll
		Chlorophyll	Carotene	Xanthophyll	
Lemon	Ringed	0.99	0.0041	0.0119	0.35
	Nonringed	1.95	0.0069	0.0165	0.42
Orange	Ringed	0.79	0.0024	0.0088	0.28
	Nonringed	1.44	0.0058	0.0093	0.62

Altogether there were 56 plants in the experiments. The experimental plants were cultivated in clay vases in the greenhouse of the Institute of Plant Physiology AN SSSR. For purposes of analysis of the ringed and non-ringed branches, primarily the leaves were tested, since it is known that leaves are very important in plant flowering [11, 12].

In lemon leaves we investigated the pigment content, changes in and content of carbohydrates and protein substance, the concentration of cell sap, and reducing capacity of the tissues. Leaves from shoots of similar age were used for analysis. As a control for the ringed shoots, we used unringed shoots of similar age from the same plant in most cases.

A significant difference was found between experimental and control branches, based on these indices.

Pigment Content

Observations of experimental plants revealed that the leaves on ringed branches gradually fade, lose their green color, and acquire a yellow color, and that these changes set in more quickly upon spring and summer ringing and more slowly when done in the winter.

Determination of the chlorophyll content in the alcoholic extracts of leaves made on the Kenig-Martens spectrophotometer showed that the chlorophyll content diminished considerably in leaves of ringed shoots. Results of chlorophyll content determination are shown in Fig. 1.

The curves in Fig. 1 show that the chlorophyll content in leaves of ringed branches diminished in relation to the time after ringing. In winter ringing (Fig. 1 A), an appreciable difference in chlorophyll content was found only 2-2½ months after ringing; in spring and summer ringing (Fig. 1 B) the difference in chlorophyll content was apparent after 2 weeks (June 25).

TABLE 2

Sugar and Amino Acid Content of Leaves of Ringed and Nonringed Lemon Shoots (in % of wet weight) (experiment of 1956)

Date of analysis	Ringed		Nonringed	
	glucose	amino acids	glucose	amino acids
June 18	0.12	0.15	0.13	0.16
June 29	0.43	0.42	0.56	1.12
August 2	0.85	0.21	1.28	0.16
August 20	0.86	0.12	0.43	0.08
September 4	0.22	0.30	0.54	0.11

TABLE 3

Reducing Capacity of Leaves of Ringed and Nonringed Lemon Shoots (1954 experiment)

Ringing date	Reducing capacity, in ml KIO_3 per g of wet weight		Ratio: Ringed
	ringed	nonringed	nonringed in %
April 14	28.9	18.1	159.7
May 8	30.2	20.4	148.0
June 1	38.4	23.2	165.5
June 25	27.8	16.8	165.5

The decrease of chlorophyll content in leaves of ringed branches proceeded more intensely in spring and summer ringing, evidently because at this period a stronger disintegration of chlorophyll takes place in relation to the increasing number of sunny days and greater intensity of illumination.

In 1955, in the third experiment, in addition to chlorophyll, the content of yellow pigments was also determined, at which time samples for analysis were taken 2 months after ringing. The separation of pigments was accomplished by column chromatography, using aluminum oxide as adsorbent. Carotene was extracted with petroleum ether, and xanthophyll by 96% ethyl alcohol with subsequent extraction by petroleum ether. Quantitative determination of carotene and xanthophyll was performed on an FEK-M photoelectric colorimeter. Results of determination of yellow pigments in leaves of ringed and unringed shoots are shown in Table 1.

The data of Table 1 show that the carotene and xanthophyll content in leaves is lower in ringed than in nonringed shoots. This indicates that in ringing not only chlorophyll, but also the yellow pigments, are destroyed.

Content of Carbohydrates and Nitrogenous Substances

The study of changes in content of carbohydrates and nitrogenous substances after ringing was conducted qualitatively and quantitatively. The starch and protein were determined initially by a qualitative color reaction directly on the leaves, and results were evaluated visually by the five ball* system. Starch was estimated by the Saks iodine test, and protein by Millon reagent, as proposed by Chailakhian [13]. Determination of starch and protein was performed on the same leaf cut in half through the mid-vein.

Numerous determinations made at different periods showed that the starch content is higher in leaves of ringed than nonringed shoots (on the average 4 1/5 units as against 2½), while the protein content, on the other hand, is higher in leaves of nonringed (4½ units) than ringed shoots (3½ units). It should be noted that the difference in starch content was considerably greater than that of protein.

The difference in the content of starch and protein, as well as in the chlorophyll content, is most marked in spring and summer ringing compared to winter ringing, and appeared much earlier. In winter ringing the determination of starch and protein content, made 20, 45, and 75 days after ringing, showed no essential differences, while in spring and summer ringing a considerable difference was found even after 2 weeks. It should be noted that the starch content in the leaves of nonringed shoots during the vegetative period underwent considerable fluctuation, but it was almost always lower than in the ringed shoots, except in those cases when the leaves from the ringed shoots were analyzed a long time after ringing, as was the case in our first experiment, results of which were published earlier [4].

* As in original – Publisher's note.

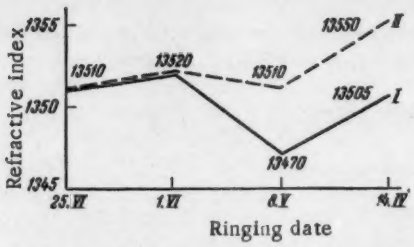

Fig. 2. Concentration of cell juice in leaves of ringed and nonringed lemon shoots (by the refractive index; in the refractive indices, the decimal is omitted) from data of experiment 2.
I) Control; II) ringed.

Data obtained by us on starch content coincide with those in the literature in indicating that ringing increases the carbohydrate concentrations above the ringed part of the plant. In addition, we believe, this will help to explain the susceptibility of chlorophyll to destruction. Chlorophyll and protein compounds break down more rapidly in leaves of ringed shoots while, on the other hand, the starch content increases. In the decomposition process the chlorophyll bond with protein is broken and a much faster fading of leaves occurs in bright sunlight. This is confirmed in part, by our determinations of chlorophyll fading in cut leaves of lemon seedlings, where the rate of chlorophyll fading and the starch content of the leaf both increased [14].

Subsequently in the experiments of 1955 and 1956, a determination was made of total and protein nitrogen content by the micro-Kjeldahl method and determination of sugars and amino acid content by the Boiarkin [15] drop method.

Quantitative nitrogen content determination has shown that there was more total as well as protein nitrogen in the leaves of nonringed than ringed shoots. This confirms our initial determinations of protein substances content, made directly on the leaf colorimetrically.

The determination of sugars and amino acids was conducted at different times. Average data of these determinations from experiment 4, all the plants of which were ringed on June 1, 1956, are given in Table 2.

The data of Table 2 indicate that in most cases the amino acid content was higher in the leaves of ringed shoots, but that the sugar content was higher in the leaves of nonringed shoots. It should be noted that the predominant sugar was glucose. Sucrose and fructose were found infrequently. They were detected in 2 instances in leaves of ringed shoots (0.21% in an analysis of August 2, and 0.22% in an analysis of September 4), and in one instance (0.32% in an analysis of September 4) in nonringed shoots.

Aside from determination of the total amino acid content, an attempt was made to separate them qualitatively by paper chromatography. On small size chromatograms development was made using isatin, as suggested by A. N. Boiarkin. We noted primarily a very high content of proline and its derivatives. A high proline content was found in lemon leaves and leaves of other citrus plants, particularly oranges and grapefruit. As regards the amino acid composition, we did not succeed in finding any differences not only in the different tests, but also in different species of citrus plants (lemons, oranges).

Altogether 17 amino acids were identified: glutathione, cystine, lysine, arginine, asparagine, aspartic acid, serine, glycine, glutamic acid, oxyproline, threonine, alanine, proline, α-aminobutyric acid, pipecolinic acid, valine, and leucine. Two amino acids among those which disappeared rapidly were not identified; in developing with isatin they are colored a light blue, the same as proline. Oxyproline, pipecolinic acid, cystine and lysine are not always found; they were isolated successfully only when applied to the chromatogram in a highly concentrated extract. The following amino acids were always found in large quantities: proline, asparagine, serine, glycine, alanine, and glutamic acid. From visual data, a large content of proline was observed in most cases in leaves of ringed shoots, and frequently there were also more of other amino acids because of a higher total amino acid content in ringed plants.

Thus, the study of the content of carbohydrate and nitrogen compounds in leaves of ringed lemon shoots and some other citrus plants has shown that in ringing, as a consequence of retarding the flow and delaying growth processes, there is an accumulation of carbohydrates, chiefly as starch, an increase of amino acid content, and a decrease of protein nitrogen content.

Reducing Capacity of Tissues

To characterize the total content of reduced compounds a determination of the reducing capacity of tissues was conducted.

The quantity of reducing substances in leaves of ringed and nonringed shoots was determined by the iodometric method by titrating the leaf extract with potassium iodate. An extract was prepared from 1 g of fresh leaves and 20 ml of 1% HCl. The leaves were ground with a small quantity of HCl. For titration purposes the following mixture was prepared: 1 ml filtrate, 2 ml 2% HCl, 1 ml 1% KI and 1 ml 0.5% starch. Titration was conducted with 0.001 N KIO_3. Determinations were conducted on plants of spring and summer ringing (experiment 2) 2-3½ months after ringing.

In all cases the reducing capacity was higher in leaves of ringed shoots, approximately 1½ times higher by comparison with nonringed ones. The data on reducing capacity are given in Table 3.

The increase of reducing capacity of leaves from ringed shoots indicates that they accumulate more reducing compounds, such as the reduced form of ascorbic acid, sulfhydril compounds, and others.

Concentration of Cell Juice

Taking into account the studies of Kolomiets [16, 17] indicating that for initial formation of fruit buds a heightened concentration of cell juices is necessary in growth cones, we conducted determinations of cell juice concentrations in leaves of ringed and nonringed lemon shoots.

Cell juice concentration was determined refractometrically and was checked by the refractive index to an accuracy of 0.0001. Average leaf samples were taken from ringed and nonringed shoots for analysis. The leaves were killed at low temperature, and subsequently the juice was pressed out by an oil press at a pressure of 150 atmospheres. Determination of juice concentration was conducted in different experiments at different periods after ringing. In experiment 1 of winter ringing the determination was conducted after 6 months, and in experiments 2 and 4 after 2 weeks or longer.

The cell juice concentration was greater in the ringed shoots in all the tests. Moreover the concentration increased with the length of time from ringing. In experiment 2 the juice concentration 1-2 months after ringing was the same in the ringed and nonringed shoots, but subsequently it became higher in the ringed shoots. Results of determination of cell juice concentration in experiment 2 are given in Fig. 2.

The data of Fig. 2 show that the concentration of cell juices is greater in leaves of ringed shoots, and that the difference in concentration between ringed and nonringed shoots sets in, depending on conditions, at various times after ringing.

SUMMARY

Ringing of separate branches of citrus plants with the purpose of acceleration of flowering and fruit formation affect the processes of growth and generative development. Due to violation of the interaction between leaves and roots the flow of plastic and physiologically active substances from the leaves to roots is retarded, the result being that growth processes are retarded and generative development processes are accelerated.

Ringing of separate branches of citrus plants strongly affects the course of metabolism in the ringed part of the plant. The leaves of ringed branches become filled with starch and dissociation of plastid pigments and proteins is intensified; this in turn leads to violation of the physiological function of the leaves. Hydrolysis processes are of dominant significance in this case: the amount of protein nitrogen decreases and the amount of amino acids increases.

Decrease of the flow of plastic substances and weakening of the interaction between the leaves and roots due to ringing lead to an increase of the reducing capacity of the leaves and an increase of the concentration of the cell sap.

In conclusion, we express deep gratitude for the guidance in the work by Professor M. Kh. Chailakhian and for his valuable advice and direction.

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INFLUENCE OF VARIOUS FORMS OF NITROGEN ON ASSIMILATION PRODUCTS OF LEAVES AND ON THEIR DISTRIBUTION BETWEEN AERIAL AND SUBTERRANEAN ORGANS IN CORN SHOOTS

S. G. Baklinova, N. G. Doman and B. A. Rubin

Institute of Plant Husbandry, Bulgarian Academy of Sciences, Sofia,

and A. N. Bakh Institute of Biochemistry, USSR Academy of Sciences, Moscow

With the adoption of techniques utilizing labeled atoms in biology, the study of transport of organic materials in plants has been greatly extended in recent years [1]. It has already been shown in the investigations of a number of workers [2-6] that sucrose is the principal sugar translocated in many plants. There is also considerable information [7] on the mechanism of translocation of other organic materials. It has been shown in numerous investigations [8-9] that roots are the organs in which a variety of synthetic processes occur. This circumstance greatly complicated our assumptions as to the interrelations of activities of the leaves and the root system and made necessary new, more extensive investigations of this problem. It is of particular interest to study the influence of mineral nutrition and light regime on the composition of the assimilation products in the leaves, etc. In this connection the work of Pristupa and Kursanov [10] is of interest. They showed that the movement of assimilates from leaves into the roots of plants suffering a nitrogen deficiency is greatly enhanced by nitrogen feeding. They believe that assimilates move into the roots, are transformed into other compounds there, and then return to the upper portions of the plant.

TABLE 1

Radioactivity of the Aerial Portions and the Roots of Corn, Expressed on the Basis of One Plant

Treatment	Light + CO_2			Dark + CO_2			Light − CO_2		
	total radioactivity in counts / min 10^3	distribution of radioactivity in %		total radioactivity in counts / min 10^3	distribution of radioactivity in %		total radioactivity in counts / min 10^3	distribution of radioactivity in %	
		stems	roots		stems	roots		stems	roots
	Four hours								
Control	1798	89.0	11.0	724	91.3	8.7	785	91.8	8.2
$NaNO_3$	1325	93.0	7.0	1010	97.0	3.0	1795	91.6	8.4
$(NH_4)_2SO_4$	1373	94.7	5.3	912	94.9	5.1	1188	95.0	5.0
	Eight hours								
Control	2368	82.0	18.0	928	91.5	8.5	907	91.0	9.0
$NaNO_3$	1890	87.3	12.7	1595	96.0	4.0	1176	92.0	8.0
$(NH_4)_2SO_4$	1483	91.0	9.0	1086	94.9	5.1	1306	91.9	8.1

However, at this time there is still much that is not clear concerning the influence which nitrogen nutrition exerts on the nature of the photosynthetic products, the translocation of assimilates and their transformations in the roots. We have devoted a series of experiments to these problems, and the results are presented in this paper.

TABLE 2

Radioactivity of Fractions Soluble and Insoluble in Alcohol from Corn Plants Maintained under Various Experimental Conditions

Treatment	Light + CO_2			Dark + CO_2			Light − CO_2		
	total radioactivity	distribution of radioactivity in %		total radioactivity	distribution of radioactivity in %		total radioactivity	distribution of radioactivity in %	
		extractable	non-extractable		extractable	non-extractable		extractable	non-extractable
				Stem, 4 hours					
Control	117000	55	45	72200	85.5	14.5	65300	51	49
$NaNO_3$	91100	48	52	79500	76	24	90200	41	59
$(NH_4)_2SO_4$	89600	54	46.5	72000	79	21	94600	41	59
				Stem, 8 hours					
Control	91700	46	54	63150	85	15	79100	29	71
$NaNO_3$	69100	36	64	83800	81	29	89500	32	68
$(NH_4)_2SO_4$	71500	39	61	86000	72	28	92600	36	64
				Roots, 4 hours					
Control	24700	94.7	5.3	11880	96.8	3.2	9300	84	16
$NaNO_3$	24080	95.4	4.6	7700	96	4	14200	75	25
$(NH_4)_2SO_4$	22200	93.5	6.5	8210	96.2	3.8	11900	76	24
				Roots, 8 hours					
Control	42500	89.5	10.5	14820	97.2	2.8	16300	85	15
$NaNO_3$	34400	91.7	8.3	9340	96.4	3.6	23950	77	23
$(NH_4)_2SO_4$	21200	87	13	11800	96.7	3.3	22600	78	22

TABLE 3

Radioactivity of the Chlorophyll Fraction (Number of counts per kg dry wt)

Treatment	Light + CO_2			Dark + CO_2			Light − CO_2		
	radioactivity of alcohol-soluble fraction	radioactivity of chlorophyll	% of radioactivity in chlorophyll fraction	radioactivity of alcohol soluble fraction	radioactivity of chlorophyll	% of radioactivity in chlorophyll fraction	radioactivity of alcohol soluble fraction	radioactivity of chlorophyll	% of radioactivity in chlorophyll fraction
				Four hours					
Control	64700	860	1.3	64200	545	0.8	34000	640	1.8
$NaNO_3$	43100	875	2.0	60000	595	1.0	36000	860	2.4
$(NH_4)_2SO_4$	53500	820	1.5	56500	540	0.95	39000	880	2.2
				Eight hours					
Control	42000	650	1.6	53700	330	0.6	30600	1280	4.0
$NaNO_3$	24600	750	3.0	60000	390	0.65	33000	1600	5.0
$(NH_4)_2SO_4$	27900	680	2.4	48000	350	0.7	35000	1560	4.4

METHODS

Corn plants were grown on nitrate and ammonium nitrogen under conditions described in a previous paper [11]. Control plants were grown in the absence of nitrogen. Plants were used from 10-15 days old, counting from the day of seed germination. The second leaf, still attached to the plant, was enclosed in a chamber containing 0.03% (according to volume) $C^{14}O_2$, and was exposed to the labeled CO_2 for 5 minutes under a 500 watt lamp 50 cm distant [12].

Following this, one group of plants in a given treatment series was left 4 to 8 house in light in an atmosphere free of labeled CO_2, and a second group was left the same length of time in the dark. The plants were

TABLE 4

Radioactivity of Various Fractions of the Alcohol Extract Obtained from the Aerial Portion of Corn

Treatment	Radioactivity of the alcohol soluble fraction	Sugars		Amino acids		Organic acids	
		in counts	in %	in counts	in %	in counts	in %
4 hours in light							
Control	64700	36850	57	22650	35	5200	8.0
$NaNO_3$	43100	27000	64	10520	24	5180	12.0
$(NH_4)_2SO_4$	53500	25200	47	23200	43.5	5100	9.5
8 hours in light							
Control	42000	25200	60.0	12600	30	4200	10
$NaNO_3$	24600	17000	69.0	4400	18	3200	13
$(NH_4)_2SO_4$	27900	17300	62.0	7500	27	3100	11
4 hours in dark							
Control	64200	36000	56	22400	35.0	5800	9.0
$NaNO_3$	60000	28800	48	22800	37.0	9000	15.0
$(NH_4)_2SO_4$	56600	23200	41	26600	47.0	6800	12.0
8 hours in dark							
Control	53700	25200	47	21400	40	6950	13
$NaNO_3$	60000	18000	30	29400	49	12000	20
$(NH_4)_2SO_4$	48000	7200	15	32000	67	8650	18
4 hours in light without CO_2							
Control	34000	24000	71	8500	25	1350	4
$NaNO_3$	36000	24500	68	10100	28	1440	4
$(NH_4)_2SO_4$	39000	20730	53	17100	44	1170	3
8 hours in light without CO_2							
Control	30600	23000	75	6100	10	1500	5
$NaNO_3$	33000	17200	52	13200	40	2600	8
$(NH_4)_2SO_4$	35000	18800	54	14100	40	2100	6

then fixed with dry steam at 100 deg for 3 minutes and dried in a vacuum desiccator. Stems and roots were separately cut up with scissors and three times extracted with boiling ethyl alcohol acidified with 1N HCl (5 ml acid and 95 ml 80% alcohol). The activity of the last extract was negligible. The extract was dried down in a vacuum desiccator over solid NaOH. The dry residue was taken up in a definite volume of water (crude solution). A sample was taken from this solution and placed on a disk for determination of radioactivity. Radioactivity was measured in a B-1 end counter. The crude solution was further fractionated in the following manner: organic acids were separated from other materials by the chromatographic method using an ethyl ether — formic acid — water mixture in the ratio 14 : 0.2 : 1.8 [13]. The portion of the chromatogram containing the organic acids was cut up and eluted three times with water on a boiling water bath. Total eluate volume was 60-70 ml. After elution no radioactivity was found on the paper. The eluate was dried down, the dry residue taken up in a definite volume of water and the radioactivity of the solution measured.

Amino acids and sugars, which remained at the origin, were eluted in the same manner as the organic acids. The eluate, containing chiefly amino acids and sugars, was treated with the cationic resin KU-1 for the removal of the amino acids. It had been established in preliminary experiments that this resin completely adsorbs all of the most abundant amino acids — neutral and acidic while the sugars remain in the solution. The radioactivity of the eluate was determined before and after treatment with the resin. The radioactivity of the so-called amino acid fraction was found as the difference between these two determinations. The radioactivity of the solution after treatment with the resin characterized the fraction made up chiefly of sugars.

The extracted plant tissues were ground in 200 volumes of water, and the radioactivity of a sample of the resulting homogeneous suspension was determined.

TABLE 5

Radioactivity of Various Fractions of the Alcohol Extract Obtained from the Roots (Number of counts per min per mg dry wt)

Treatment	Radioactivity of the alcohol soluble fractions in counts per min	Sugars		Amino acids		Organic acids	
		in counts	in %	in counts	in %	in counts	in %
		4 hours in light					
Control	23400	18700	80	2700	11.5	2000	8.5
$NaNO_3$	16300	12200	75	3400	21.0	650	4.0
$(NH_4)_2SO_4$	11400	8000	70	2700	24.0	680	6.0
		8 hours in light					
Control	38000	25800	68	9100	24.0	3400	8.0
$NaNO_3$	31500	18900	60	11200	35.5	1420	4.5
$(NH_4)_2SO_4$	18200	10400	57	6900	38.0	900	5.0
		4 hours in dark					
Control	11500	7350	64	2700	18	2700	18
$NaNO_3$	7400	4150	60	1850	25	1100	15
$(NH_4)_2SO_4$	7900	4900	62	2200	28	790	10
		8 hours in dark					
Control	14400	9000	63	4200	29	1150	8
$NaNO_3$	9000	6100	56,5	2980	33	950	10
$(NH_4)_2SO_4$	11400	5150	45	4900	47	900	8
		4 hours in light without CO_2					
Control	6800	3000	45	2220	33	1500	22
$NaNO_3$	10500	4000	38	4000	38	2500	24
$(NO_4)_2SO_4$	8900	2700	30	4200	47	2000	23
		8 hours in light without CO_2					
Control	9800	5400	55	3100	32	1280	13
$NaNO_3$	18500	9600	52	5700	31	3600	17
$NH_4)_2SO_4$	17100	8200	48	7200	42	1700	10

To determine the radioactivity of the chlorophyll, the alcoholic extract was filtered through filter paper after standing 24 hours. After a washing with alcohol, flakes of pheophitinized chlorophyll were evenly distributed on the filter, and the filter was then dried and flattened out, and the radioactivity of the thin film which had formed directly measured over the whole area of the filter in an end counter.

RESULTS

In Table 1 are presented data on the distribution of radioactivity in corn plants after 5 minutes of photosynthesis in the presence of $C^{14}O_2$ with a subsequent period of 4 to 8 hours in the light or the dark.

In the left portion of Table 1 (light, dark) it can be seen that radioactivity in the roots is higher in the light than in the dark. This difference is especially evident 8 hours after exposure to labeled CO_2.

The difference in radioactivity of roots of plants kept in the light and the dark testifies to a favorable influence of photosynthesis on translocation of materials to the roots. In order to clarify whether this hastening of translocation is dependent on light or on the accumulation of photosynthetic assimilates, we set up an additional experiment in which following exposure to $C^{14}O_2$, the plants were placed in a lighted chamber through which air free of CO_2 was passed.

The right portion of Table 1 (light) shows that after plants have been kept for 8 hours in the light in an atmosphere free of CO_2, the activity of the roots is intermediate between that of roots of plants kept in the light and in the dark in the presence of CO_2. This is explained by the fact that under these conditions photosynthesis is not completely abolished, but continues at the expense of labeled CO_2 given up by the plants in respiration [15]. Nevertheless, we can conclude on the basis of these data that the existence in the leaves of sufficient quantities of photosynthetic assimilates is of basic importance in the translocation of materials.

In a comparison of plants grown on the different forms of nitrogen with plants suffering a nitrogen deficiency it is seen that in the light in the presence of CO_2, as well as in the dark, translocation into the roots of plants deficient in nitrogen is more rapid than in plants supplied with nitrogen. It should be noted that in plants grown without nitrogen the root system is considerably more developed and the total length of their roots is greater than in plants grown with nitrogen [11]. Moreover, the heightened movement of labeled assimilates into roots is associated with this. The increased development of the root system may in all probability be regarded as an adaptive reaction to an unfavorable regime by 10-15-day-old plants.

In plants grown on nitrate nitrogen and kept in the light, the quantity of assimilates entering the roots is somewhat higher than in plants grown on ammonium nitrogen. The opposite situation exists with plants kept in the dark.

In a comparison of the data in Table 1 expressing the total radioactivity with the data of Table 2 expressing the radioactivity of fractions soluble and insoluble in 80% alcohol, the same patterns are evident, although they are clearer in Table 2.

It can be seen in Table 2 that the amount of material not extractable by alcohol is two times greater in the aerial parts of plants kept in the light than in those of plants kept in the dark. This indicates that synthesis of materials of high molecular weight insoluble in alcohol — starches, celluloses and proteins — is more rapid in the light than in the dark. In the light in an atmosphere free of CO_2, the greatest quantity of materials insoluble in alcohol is formed. Evidently this is due to the fact that synthetic processes involved proceed under these conditions at the expense of labeled CO_2 released by the plants in respiration, and dilution of the isotope is less extensive.

In the roots, the proportion of radioactivity found in the insoluble fraction of plants kept in an atmosphere containing CO_2, both in the light and the dark, is insignificant (3-13%) as compared with the radioactivity of the soluble fraction. The increased percentage of radioactivity of this insoluble fraction in light and in the absence of CO_2 commands attention.

The influence which the nitrogen regime exerts on chlorophyll synthesis is of interest (Table 3).

As Table 3 shows, the percentage of radioactivity associated with chlorophyll is very small. With respect to the distribution of radioactivity in the chlorophyll, the same pattern as with the insoluble fraction was found, namely, in the light in the presence of CO_2 the radioactivity of this fraction is higher than in the dark. In the light without CO_2 there was an increase to 5% of the activity of the chlorophyll fraction. This difference is apparently due to the fact that in the light in the presence of unlabeled CO_2 there is a continuous dilution of previously labeled photosynthetic products, while in the light in the absence of CO_2, photosynthesis proceeds only at the expense of the partially labeled CO_2 produced in respiration, as was previously pointed out. As a result, more highly labeled products are obtained, which is especially evident with such comparatively immobile materials as starch and chlorophyll. Table 3 shows also that the activity percentage in chlorophyll of plants grown on nitrate is somewhat higher than in controls and plants grown on ammonium nitrogen. This agrees with the data obtained with Rubin and Vaklinova [11] showing that in plants grown on nitrate nitrogen there is more chlorophyll than in plants grown on ammonium nitrogen.

Some interesting data were obtained in an investigation of the alcohol soluble fraction (Table 4).

In Table 4 it can be seen that the greatest radioactivity in the light is in the fraction consisting principally of sugars (47-67%). A perceptibly lower activity is found in the amino acid fraction (17-44%), and a comparatively weak activity (7.5-13%) in the organic acid fraction. With 4 hours in the dark, as in the light, the activity of the sugars is high, but it gradually decreases, and after 8 hours it is less than that of the amino acids, whose content increases. The decline in sugar level in the dark is probably associated with their degradation in respiration, as well as with their conversion into other labeled products such as organic acids and amino acids. The percentage of radioactivity in the organic acids has an overall tendency to increase in the light and particularly in the dark.

The higher level of amino acids in the dark is apparently associated with a lower rate of utilization in protein synthesis in leaves as compared with the rate at which this occurs in the light, as shown by Andreeva and Naborchik [15].

In comparing plants subjected to various nitrogen regimes, it can be seen that in the light the largest amount of sugar is accumulated in plants grown on nitrate nitrogen, while in the dark the most sugar is found in the controls. It should also be noted that in the light on nitrate, when protein synthesis is more rapid [16], the lowest percentage of free amino acids is found. In the dark on ammonium nitrogen we observed the lowest sugar content and the highest free amino acid content in comparison with the control and with plants grown on nitrate nitrogen.

On nitrate nitrogen in the light as well as in the dark the highest level of organic acids is found, which agrees with published data [17-19]. With an increase of the exposure time to an atmosphere free of CO_2 in the light, there is little change in the radioactivity of sugars and amino acids, expressed as percentage of the radioactivity of the soluble fraction.

Comparing the data of Tables 4 and 5 (see Table 5), it can be concluded that the relative content of labeled sugars in corn roots is substantially higher than in aerial parts.

With an increase of the exposure time in light, the radioactivity of sugars in the roots, expressed as a percentage of the radioactivity of the alcohol soluble fraction, declines, while there is a simultaneous rise in amino acid activity. The activity of the organic acids in the roots of illuminated plants, as in the stems, does not change essentially under the conditions of our experiments. In the dark radioactivity of sugars is somewhat lower than in the light; with an increase in exposure time it declines. Associated with this is an increase in the activity of the amino acid and organic acid fractions both in the aerial parts and the roots.

The higher sugar content of the roots as compared with the aerial portions is probably due to the fact that sugars are the principal form in which organic materials are transported from the leaves to the subterranean organs. The decreased sugar levels in the roots of plants kept in the dark are in all probability associated with a lowered rate of translocation of products from the leaves to the roots as well as with a more rapid rate of utilization in respiration. The increase in organic acid content is also related to the increased respiratory activity.

With the absence of nitrogen from the nutrient environment, the ratio between sugar activity and amino acid activity is shifted somewhat toward the sugar component, while on nitrate and ammonium nitrogen it is shifted toward the amino acid component. This is in good agreement with all published data [20].

It is noteworthy that in plants kept in an atmosphere free of CO_2 in the light, the percentage of labeled organic acids is high and that it decreases markedly at least in 8 hours; this decrease, with one exception, is also expressed in the absolute radioactivity levels.

SUMMARY

Leaves of intact maize shoots grown under various conditions of nitrogen nutrition assimilated $C^{14}O_2$ during a 5 minute period after which the plants were grown under a variety of conditions.

The nitrogen absorbed by plant roots significantly influences the chemical composition of the photosynthetic assimilates and their movement to the roots. In all cases plants grown in the absence of nitrogen accumulated more radioactivity in the roots than plants supplied with nitrogen. Due to accelerated translocation of assimilates in light the total activity in the roots was found to be higher than when the plants were in the dark. In plants illuminated in an atmosphere without CO_2 the flow rate of the assimilates was intermediate between that observed in illuminated and in darkened plants in the presence of CO_2. This signifies that enhancement of the rate of flow of assimilates to the roots in illuminated plants is caused by the larger amount of the assimilates in the leaves.

In all cases the radioactivity of the aerial part of the plants was several times higher than in the roots. The fraction of assimilates which could not be extracted by alcohol from aerial organs of the plants was significant (up to 65%), whereas in roots most of the assimilates could be found in the alcohol solution. In aerial organs of illuminated plants the fraction which could not be extracted with alcohol exceeded by more than two times that from dark plants.

Assimilates produced during photosynthesis change in the subsequent dark period into products which are characteristic of dark fixation of CO_2 (decrease of sugar content, accumulation of amino acids and organic acids) whereas in light the pattern is the same as that observed in the case of photosynthesis.

The composition of the products of CO_2 assimilation and their transformation in the aerial organs and roots under light and dark conditions depend on the form of the nitrogen supplied.

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STUDY OF GROWTH PROCESSES IN WINTER RYE VERNALIZED AT TEMPERATURES BELOW ZERO DEGREES CENTIGRADE

V. G. Grif

All-Union Institute of Plant Husbandry, Leningrad

It has been established by the work of many investigators [1-5] that the rate of vernalization in winter cereals does not depend on the growth rate of the plants. There are, however, indications that a minimal growth is necessary during vernalization [6, 7].

The published data on the lower temperature limit at which growth processes are possible in winter cereals are contradictory. The majority of workers [8-11] believe that it is about 0°. Certain authors [12 and others], however, assert that growth of winter cereals is possible at still lower temperatures (below 0°). With respect to physiological processes, many of them may proceed in winter cereals at temperatures considerably lower than 0° according to published information. Specifically, it has been shown by many workers [13-24] that vernalization may take place in these plants at sub-zero temperatures. It is still unclear whether growth processes were observed during this time or whether vernalization occurs without them.

For a clarification of this problem a cytological investigation of growth processes (universally associated with cell division in meristematic tissues) in the embryos of grains vernalized at sub-zero temperatures was undertaken. The plant used was winter rye, variety Viatka. Experiments were performed under the direction of T. Ia. Zarubailo, candidate in biological sciences.

There are indications in the literature that it is possible to halt cell division at any given mitotic stage by very rapid freezing of the dividing cells [25, 26]. With such frozen material which has been fixed and further treated by the usual cytological method it is not possible to establish just when the mitotic figures observed in the preparations arose — before freezing or as a result of cell division at sub-zero temperatures. Therefore, in 1952 a preliminary experiment was carried out to determine the times of beginning of cell division in a grain during germination under controlled conditions. The experiment showed that when the seeds were imbibed and germinated at 20° cell division in the rootlets of an embryo of rye began after 28-30 hours (depending on the rate of seed germination), in the leaves — after 38-40 hours, and in the terminal meristem of the stem — after 42-44 hours.

Using this information, we developed a method of determining the upper limit of the minimal temperature, at which cell division of a germinating embryo just ceases. Taking into account the time of beginning of cell division in various parts of the embryo under definite germination conditions, we permitted germination to proceed for such periods prior to freezing that after the first germination period (28 hours), the cells of the embryo were ready to divide, but had not begun to do so, after the second germination period (41 hours) — division had begun in the rootlets and leaves and, finally, after the third period (66 hours) — it had begun in all parts of the embryo including the terminal meristem of the stem.

At the end of each of the germination periods the seeds were placed in refrigerators at 0, −2 and −3°. At the same time some of the seeds were fixed as controls. All seeds were kept in the refrigerators for 77 days. On the 28th and the 77th day of vernalization samples were taken and were treated by the standard cytological method for preparation of permanent material. The sections were 8-12 μ thick; the material was stained by the Schiff reaction as modified by Feulgen. By comparing control material fixed at the time of freezing with material fixed on the 28th and 77th days of vernalization, the temperature at which cell division ceased was determined.

TABLE 1

Presence of Mitoses in Embryos of Seeds Vernalized at 2° (percentage of total number of embryos studied)

Germination period prior to vernalization (in hours)	Time of fixation*	With prophases			With metaphases			With anaphases			With telophases		
		in rootlets	in leaves	in terminal meristems	in rootlets	in leaves	in terminal meristems	in rootlets	in leaves	in terminal meristems	in rootlets	in leaves	in terminal meristems
28	I	7	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0
41	I	89	80	0	89	50	0	89	40	0	89	30	0
	II	100	50	0	90	30	0	90	30	0	70	20	0
	III	86	71	0	86	43	0	86	43	0	86	43	0
66	I	100	100	100	100	100	100	100	100	100	100	100	100
	II	100	100	100	100	100	100	100	100	100	100	100	100
	III	100	100	100	100	100	86	100	100	86	100	100	86

* I) Control (before vernalization); II) on the 28th day of vernalization; III) on the 77th day of vernalization.

TABLE 2

The Effect of a Temperature of −2° on Cell Division in Rye Embryos (average number of mitoses in a field)

Time of fixation	In roots	In leaves	In the terminal meristem
Control (before freezing)	7.14	6.68	6.01
Experimental (on the 77th day of vernalization at −2 deg	6.69	6.01	5.88

Using this method we carried out in 1953 and 1954 a cytological study of division processes prior to vernalization at sub-zero temperatures. Because of space limitations we will limit ourselves to a consideration of the data of 1954, the results of 1953 being in agreement with them.

A study of control preparations, fixed at the time of the experimental lots were placed at 0°, showed that the embryonic tissues were ready to divide since prophases were observed in rootlets of various embryos. On the 16th day, all the mitotic phases were observed in the rootlets. Only preparations fixed on the 48th day of vernalization showed these phases in the leaves and the terminal meristem.

It may therefore be concluded, that at 0° winter rye can grow, though very slowly. No differences in the behavior of the various parts of the embryo at 0° were noted. The later beginning of cell division in the leaves and the terminal meristem is explainable in terms of the fact that even at optimal conditions these organs begin growth 12-16 hours later than the rootlets. With a gradual lowering of the temperature to 0° or lower, mitotic activity disappears, i.e. the dividing cells complete their division cycles, but new mitoses do not arise. As a result the cells of the plant pass into a resting condition with the appearance of cold. This condition is a protection against unfavorable conditions of winter [27, 28].

At a temperature of about −2° and lower there is a cessation of division processes of whatever phase the cell happened to be in at the time of freezing. The cells remain in this "fixed" condition throughout the entire freezing period. From the data presented in Table 1 it is clear that at −2° the percentage of embryos in which cell division has begun in the various organs remains unchanged during the entire vernalization period. Thus, in the first group (with a 28-hour germination period prior to vernalization), cell division was observed neither in

the control embryos nor in the embryos fixed on the 28th and 77th days of vernalization, with the exception of prophases in certain rootlets. In the second group, mitoses were found in the rootlets and leaves of the controls, prophases being present in almost all preparations and telophases only in certain ones. Analogous observations were made with material fixed on the 28th and 77th days of vernalization. Finally, in the third group cell division was almost completely under way in all the organs of the embryos. This situation was observed throughout the vernalization period. Consequently, cell division in rye at −2° cannot begin, and divisions already under way are "fixed" at this temperature.

In order to show conclusively that with a rapid lowering of the temperature to −2° all mitoses are "fixed", a count of the number of mitoses in the microscope field was made. The count was made on control preparations and on preparations fixed at 77 days, the end of the vernalization period. Counts in various organs of the embryo were made at a magnification of 124 x 10 (immersion). Ten preparations in each group were counted. The results are presented in Table 2.

Table 2 shows that the number of mitoses remained unchanged throughout the experiment. Therefore, with rapid freezing all mitoses were "fixed", since it is impossible that growth occured at the same rate at −2° as at +20°, at which the initial germination took place.

This technique may probably be used in the determination of the mineral temperature at which growth of green plants, including woody plants, is possible. By comparing the average number of mitoses in similar preparations fixed before freezing and at definite times after freezing, it is possible to determine the temperature at which there are no changes in the number of mitoses in meristematic tissues. That temperature at which the number remains unchanged will then be the temperature at which plant growth ceases.

A preliminary vernalization at 0° for several days (in the experiment, 16 days) does not increase the capacity of plants to grow at sub-zero temperature. In this case cell division is also stopped at that stage which prevailed at the time of freezing.

When seeds vernalized at 0° were sown, they gave plants all of which produced upright stems 20 days and headed 38 days after the appearance of seedling shoots. No difference in the times at which these physiological events occurred was ever found between groups with different germination periods prior to vernalization.

An analysis of plants obtained from seeds vernalized 77 days at −2° which was performed on September 15 showed that, notwithstanding the absence of growth at this temperature, vernalization occurred. In this group 96% of the plants produced upright stems, and of these 68% headed; the inception of these stages occurred only 6-7 days later than with plants from seeds vernalized at 0°. The data obtained indicated that at sub-zero temperatures vernalization is much slower. These results are in complete agreement with published information [29]. An analogous pattern was observed when seeds vernalized at 0° for 16 days and subsequently at −2° for 53 days were sown. In this experiment 98% of the plants produced upright stems and 89% headed. In no case was there a difference in the time at which these events occurred between groups with differing germination periods prior to vernalization, i.e. between groups which had begun growth at the time of vernalization and those in which no cell division had been observed throughout the vernalization period.

Thus it is obvious that sequential changes of vernalization may occur in plants whose growth processes have been stopped. Moreover, imbibed seeds in which the meristematic tissue of the embryo is ready to divide but in which division has not yet begun to undergo vernalization at sub-zero temperatures. It is probable that even under conditions optimal for vernalization of seeds embryo growth is not obligate. It may be supposed that during vernalization at 0° to −2° with a limited water supply there are also no growth processes, since in vernalization, even at the end of the vernalization period, the seed coat is not ruptured by the primary rootlet of the embryo. As our observations indicated, division in the rootlets is begun only after rupture of the seed coat, and in the other parts of the embryo even later. It may therefore be supposed that with limited moisture supply only a physiological activation of the tissues takes place, and growth processes are not begun, particularly in the terminal meristem of the stem. This supposition requires, of course, experimental confirmation.

SUMMARY

Cell division in different organs of the embryo does not take place simultaneously; it begins first in the rootlets, then in the leaves and, finally in the terminal meristem of the shoot. Under optimal growth conditions

the difference between the beginning of cell division in the rootlet and in the apical cone is from 12 to 16 hours.

The minimal growth temperature for winter rye is somewhat lower than 0° C but above −1° C. At this temperature the growth rate is extremely small.

Quick freezing of growing plants terminates growth processes (stops cell division, their elongation). All growth processes are "fixed" at the stage they reached when the negative temperature set in.

With rapid lowering of the temperature to −2° C or lower all growth processes are "fixed", no noticeable difference in the reaction of various organs of the plant to quick freezing being observed. Preliminary vernalization several days before freezing at a temperature of 0°C does not increase the ability of the plant to grow at low temperatures.

It is not possible to conclude that growth occurs at sub-zero temperatures on the basis of mitoses observed in preparations of material "fixed" at these temperatures, as in this case in the tissues of the plants there may always be a certain amount of mitotic activity which was "fixed" by the abrupt fall in temperature. This may lead one to the false conclusion that growth is possible even at very low temperatures.

Growth is inhibited at temperatures somewhat below 0° C but vernalization processes continue. Vernalization can take place unaccompanied by growth processes.

Swelled seeds which have not yet begun to germinate (i.e. cell division has not begun) can be vernalized at temperatures below 0° C; the same holds for germinating seeds whose growth was interrupted by freezing. The rate of vernalization is independent of the mitotic activity of the plant tissues before freezing.

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EFFECT OF NITROGEN AND PHOSPHORUS FERTILIZERS ON SOME PHYSIOLOGICAL PROCESSES AND PRODUCTIVITY OF THE COTTON PLANT

N. S. Petinov and Kh. Samiev

K. A. Timiriazev Institute of Plant Physiology, Academy of Sciences USSR, Moscow, and Institute of Genetics and Plant Physiology, Academy of Sciences USSR, Tashkent

Mineral elements occur in plants as ions, free or absorbed, or as complicated organic substances [1]. The presence of mineral salts in cells greatly affects the structure of biocolloids, their hydration, and the fractional water content, which, in turn, changes the physiological-biochemical processes occurring in plants [2]. Because of this, the interrelationships of the concentration of mineral salts represent one of the most important regulators of these processes.

A more favorable water regime of leaves may be created under the influence of corresponding mineral salts, which may then be characterized by the optimal interrelationship of free and bound water in leaves, a higher degree of colloid hydration at relatively low osmotic pressure. All this, taken together, determines the physiological activity of plants, their growth and productivity [3]. A high degree of colloid hydration is, in addition, the most important factor in the aggregate stability of the colloidal system of protoplasm, and, consequently, in the plant's resistance as a whole to unfavorable conditions.

In spite of the great importance of the questions mentioned above, the study of the changes in leaf water regime in plants growing under different conditions of mineral nutrition has only recently been undertaken [2, 4]. This is a very important circumstance, since in the literature up to the present time we find only isolated communications concerning the effect of ions of various mineral nutrient salts on the state of water in a plant. In particular, it has been pointed out that phosphate fertilizers increase the water-holding capacity of protoplasmic colloids through an increase in the amount of bound water [5, 6]. The nitrate salts, however, decrease the amount of colloid-bound water, but the total amount of water in the leaf is positively correlated with nitrogen and phosphorus [7]. More detailed investigations have shown, however, that the action of various fertilizers on the change in the fractional composition of water in plant leaves varies according to age. It was found that early supplementary feeding with superphosphate increased the amount of bound (total amount) and colloid-bound water in leaves. A later supplementary nitrogen feeding had an analogous result. Later supplementary feeding with phosphorus and early feeding with nitrogen had only a weak effect. The greatest effect was obtained with combined supplementary feeding (early phosphorus and late nitrogen feeding). The analysis of the causes which induce an increase in colloid-bound water in plant leaves, under the influence of mineral supplementary feeding, has shown that the growth depends to a small degree on the increase in dry weight of colloids, and to a large degree, on the values of the hydration numbers of colloids. One may refer to the data of experiments in which solutions of nitrate salts were injected, with the result that osmotic pressure was increased [2]. With phosphorus supplementary feeding, osmotic pressure was almost the same as in the control. The authors believe that the cause of this lies in a great solubility of nitrates in water, and in an increase in the osmotic pressure of the soil solution. But the increase in osmotic pressure in leaves in these cases was temporary in nature. This is very important, since an increase in osmotic pressure above a certain level over a long period leads to a decrease in the hydration rate, to a decrease in the amount of colloid-bound water, and, on the contrary, to an increase in the amount of osmotically bound water [2].

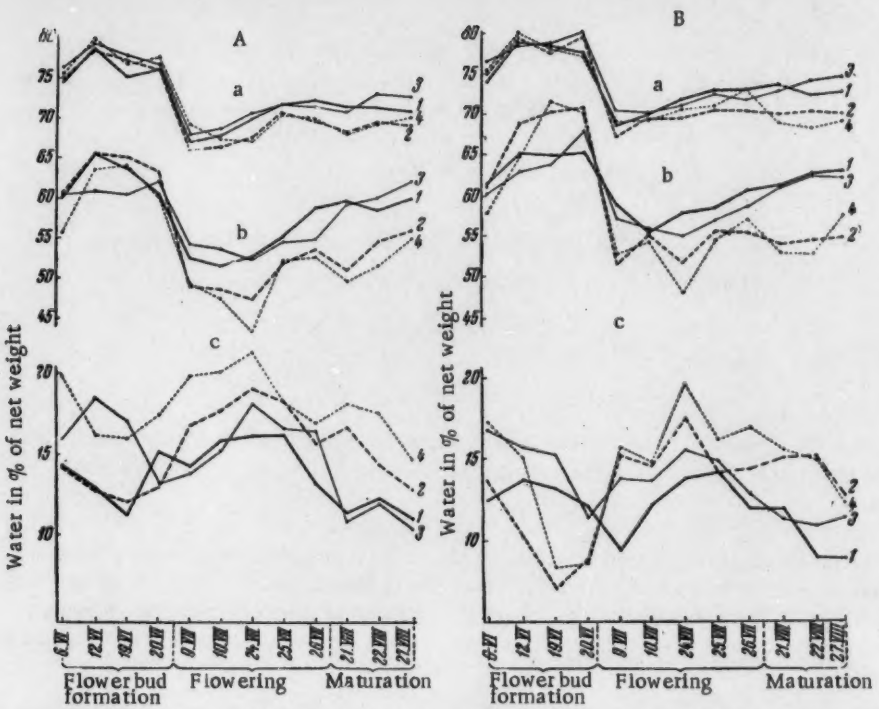

Fig. 1. Forms of water in leaves of cotton, variety 108-F, at various soil moistures and different mineral nutrition.

A) At 40% soil moisture; B) at soil moisture of 65% of full field capacity: a) total water content; b) free water content; c) bound water content. 1) Control without supplementary feeding; 2) supplementary feeding with nitrogen; 3) supplementary feeding with phosphorus; 4) supplementary feeding with nitrogen and phosphorus.

TABLE 1

Effect of Mineral Nutrition on Ratio of Free to Bound Water in Cotton Leaves at Various Soil Moisture Levels (experiment of 1957)

Date	Stage of plant development	Soil moisture in percent of field capacity							
		65%				40%			
		O	N	P	N P	O	N	P	N P
6.VI	3-4 true leaves	4,91	4,47	3,82	3,35	4,2	4,29	3,75	2,82
12.VI	Beg. of flower-bud formation	4,71	6,6	4,03	4,29	5,06	5,11	3,3	3,9
19.VI	Flower-bud formation	4,87	9,77	4,23	8,2	5,62	5,35	3,54	5,3
20.VI	Flower-bud formation	5,31	8,53	5,85	7,77	3,97	4,89	4,7	3,45
9.VII	Beg. of flowering	6,14	3,33	4,06	3,32	3,65	2,89	3,86	2,46
10.VII	Beg. of flowering	4,62	3,7	4,01	3,62	3,22	2,72	3,44	2,31
24.VII	Flowering	4,1	2,9	4,07	2,5	3,23	2,47	2,88	2,1
25.VII	Flowering	4,03	3,85	3,77	3,31	3,36	2,84	3,3	2,84
26.VII	Flowering	4,89	3,79	4,46	3,6	4,38	3,39	3,29	3,08
21.VIII	Maturation	4,93	3,4	4,28	3,33	5,14	3,02	5,36	2,71
22.VIII	Maturation	6,56	3,47	5,44	3,42	4,65	3,75	4,9	2,9
27.VIII	Maturation	6,56	4,39	5,19	4,65	5,52	4,3	6,05	3,68

As the concentration of the cell sap is increased above a certain level due to the action of nitrate salts, the synthetic processes, particularly protein synthesis, are retarded [8]. All this leads to a deterioration of the

TABLE 2

Effect of Mineral Nutrition on Amount of Osmotically Bound Water in Leaves of Cotton Plant 108-F at Various Soil Moisture Levels (experiment of 1957)

Date	Stage of plant development	Soil moisture in % of field capacity							
		65%				40%			
		O	N	P	NP	O	N	P	NP
June 20	Flower-bud formation	6.1	6.24	6.0	6.2	6.62	6.84	6.84	7.04
July 9	Beginning of flowering	7.42	8.0	8.12	8.0	8.3	8.8	8.2	8.67
July 10		7.6	7.9	7.7	8.6	8.5	8.4	8.4	8.8
July 24	Full flowering	6.8	7.8	6.8	7.9	8.0	8.6	8.5	8.6
July 26		7.74	7.9	7.7	8.67	8.4	8.92	8.67	8.97
August 21	Beginning of boll maturation	7.04	8.4	7.9	8.2	8.67	9.32	8.2	9.4
August 22		7.6	8.67	8.4	9.0	8.12	9.22	8.5	9.3
August 27	Period of boll opening	6.62	8.8	8.1	8.8	8.0	9.22	7.42	9.1

TABLE 3

Change in Osmotic Pressure (in atmospheres) and Cell Sap Concentration (in % of dry weight) in Leaves of Cotton Plants Given Nitrogen and Phosphorus Fertilizers on a Background of Various Soil Moisture Levels

Date	Stage of development	Soil mosture in % of field capacity							
		65%				40%			
		O	N	P	NP	O	N	P	NP
	Osmotic pressure								
31.V	4-5 true leaves	6,87	—	—	—	10,12	10,68	10,96	10,51
19.VI	Flower-bud formation	12,0	14,68	12,28	14,0	12,72	14,44	12,76	15,08
9.VII	Beg. of flowering	13,0	15,7	13,6	14,6	12,6	16,0	13,8	17,0
24.VII	Flowering	16,16	19,0	17,56	19,72	18,24	21,04	18,76	22,76
22.VIII	Maturation	17,08	21,16	17,08	18,04	18,28	24,04	16,38	23,32
27.VIII	Beg. of boll opening	14,68	—	15,64	20,92	13,96	22,84	17,8	—
	Cell sap concentration								
31.V	3-4 true leaves	7.0	7.4	7.0	7.1	7.8	8.1	7.8	7.8
20.VI	Flower-bud formation	10.1	10.4	10.0	10.2	10.8	11.2	11.2	11.6
9.VII	Beg. of flowering	12.4	13.3	13.6	13.4	13.9	15.2	14.1	15.0
10.VII		12.6	13.2	12.8	14.8	14.3	14.8	14.4	15.4
24.VII	Full flowering	11.2	13.0	11.2	13.2	13.4	14.9	14.6	14.8
26.VII		12.8	13.2	13.2	15.2	14.4	15.8	15.2	15.9
21.VIII	Period of maturation	11.6	14.4	13.3	14.0	15.0	16.8	13.9	17.0
22.VIII		12.7	15.1	14.3	16.1	13.7	16.3	14.5	16.7
27.VIII	Period of boll opening	10.8	15.4	13.4	15.6	13.4	16.4	12.4	16.0

water regime of plants. Here, one can conclude that the main action of mineral salt ions, which get into leaves, will consist, as already mentioned, in the increase of the degree of hydration of the protoplasmic colloids.

The degree of colloid hydration and the amount of colloid-bound water, closely connected with it, have a determining effect on the most important physiological processes. Thus, the rate of transpiration is inversely dependent, and of photosynthesis, directly dependent, on the amount of colloid-bound water. Because of this, by affecting the degree of colloid hydration and the amount of colloid-bound water by mineral nutrition, we can change the rates of transpiration and of photosynthesis.

Of great interest are the experiments which indicate the possibility of a decrease in transpiration and a considerable increase in yield in a number of agricultural crops through application of mineral fertilizers (N, P, K, and Ca) in optimal amounts, established by the so-called wilting method [9-14].

TABLE 4

Limits of Variations of Different Forms of Nitrogen and Phosphorus Throughout Growing Season of 1957 (in mg per 100 mg dry weight)

Indicators	Soil moisture in % of field capacity			
	65%			
	O	N	P	NP
Nitrogen				
Total	2.22—3.95	3.36—4.87	2.33—3.97	2.70—5.19
Protein	2.19—3.57	2.51—4.13	2.15—3.79	2.1 —4.77
Nonprotein	0.03—0.58	0.45—1.27	0.15—0.55	0.274—1.09
Phosphorus				
Total	158—358	88—337	197—457	100—336
Protein	35—200	22—134	60—254	30—174
Nonprotein	120—215	74—209	74—223	64—216

Indicators	Soil moisture in % of field capacity			
	40%			
	O	N	P	NP
Nitrogen				
Total	2.04—4.34	3.37 —4.74	2.54—4.04	3.12—5.43
Protein	1.76—3.72	2.33 —4.38	2.18—3.88	2.69—4.69
Nonprotein	0.12—0.61	0.355—1.04	0.24—0.69	0.38—0.84
Phosphorus				
Total	118—340	98—333	264—427	158—429
Protein	52—157	23—139	125—196	38—134
Nonprotein	75—176	65—198	105—260	112—294

Unfortunately, we find almost no data in the literature on the effect of mineral nutrition on the above-mentioned complex of indicators of the water regime of cotton plants, in connection with its productivity, although many questions on the physiology of the water regime of this crop [4], as well as the basis for a rational watering regime, are well developed [14-17].

The establishment of the interrelationship of these indicators, characterizing the rate and direction of physiological processes in cotton plants, makes it possible to seek a way to increase cotton productivity. The present paper is the first stage in this direction.

Vegetative experiments were carried out in 1955 and 1956 in the Institute of Genetics and Plant Physiology of the Academy of Sciences USSR (Tashkent).

METHODS

As experimental material, we used cotton plants, variety 108-F, grown in vegetative vessels holding 26 kg of soil. The soil in the vessels was typical heavily argillaceous sierozem, with deep location of soil water. The soil was mixed with sand in a ratio of 3 : 1 before filling vessels.

The experiment consisted of four treatments, along the following plan: 1) without fertilization (control); 2) nitrogenous fertilization; 3) phosphorus fertilization; 4) fertilization with nitrogen and phosphorus.

The experiments were carried out at two water regimes: 40% and 65% of the full field capacity, maintained by daily or twice-daily watering, by weight.

The fertilization used was: phosphorus as 18% superphosphate, and nitrogen as 34% ammonium nitrate. One g N and 1 g P_2O_5 were applied when the vessels were filled, and four supplementary feedings of 1 g each of

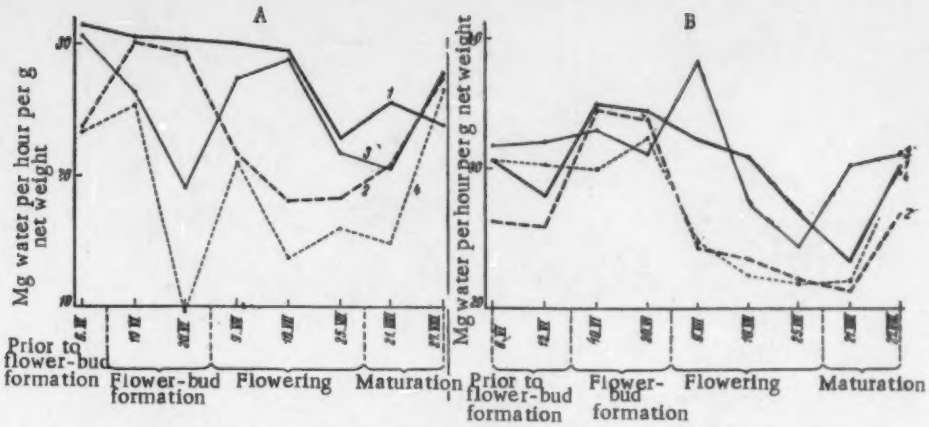

Fig. 2. Transpiration rate in leaves of cotton, variety 108-F, under the influence of different soil moisture and mineral nutrition conditions.
A) At 40% soil moisture; B) at 65% soil moisture; 1) control without supplementary feedings; 2) supplementary feeding with nitrogen; 3) supplementary feeding with phosphorus; 4) supplementary feeding with nitrogen and phosphorus.

N and P_2O_5 were applied later (at the stage of 3-4 true leaves, during flower-bud formation at flowering, and at the beginning of maturation). The total level of fertilizers per vessel was, then: N - 5 g, P_2O_5 - 5 g.

In 1956, the experiments were carried out in 10 replicates, and in 1957, in 25 replicates.

The plantings were carried out at the usual times — during the second decade of April. We have determined the following: 1) fractional composition of water — a) total water content, by drying to constant weight at 105° C, b) free water, by Marinchik's method [18], c) bound water, by difference between total and free water, d) osmotically bound water, according to the hydration of the cell sap. Since the hydration of the cell sap is close to the hydration of sucrose in isotonic concentration, then this hydration, expressed in percent of the total water reserve, represents the amount of osmotically bound water, e) colloid-bound water, by the difference between the total amount of bound water and the amount of osmotically bound water; 2) osmotic pressure of the cell sap, by the cryoscopic method; 3) concentration of cell sap, by means of a Zeiss refractometer; 4) forms of nitrogen, total by Kjeldahl, protein by Barnshtein; 5) phosphorus, by the method of Fiske-Subbarow; 6) transpiration value, by means of weighing vessels with plants, and parallel sets without plants; 7) transpiration rate, by the weight method of Ivanov.

EXPERIMENTAL PART

Let us consider the data on the effect of mineral fertization on the content of different forms of water in leaves of the cotton plant under different water regimes (Fig. 1).

From the data of Fig. 1, it is easy to see that there was no significant difference in the total water content in the leaves at the early stage of development (4-5 true leaves and in the phase of flower-bud formation).

At a later stage (flowering), a decrease in the total water content was observed in leaves of cotton plants given supplementary feeding with nitrogen and with nitrogen plus phosphorus.

In treatments with supplementary feeding of phosphorus, on the contrary, an increase in total water content was observed, as compared with the controls.

There was less free water at the stage of flower-bud formation in the leaves of control unfertilized plants, and in those fertilized with phosphorus, as compared with those treatments which got nitrogen and nitrogen-phosphorus supplementary feedings. Futher, in the later stages of development the picture was reversed (Fig. 1).

Mineral fertilizers also had an effect on the content of bound water in the leaves of the cotton plant, increasing its amount. In this case too, nitrogen and nitrogen-phosphorus fertilizers at the flowering stage were

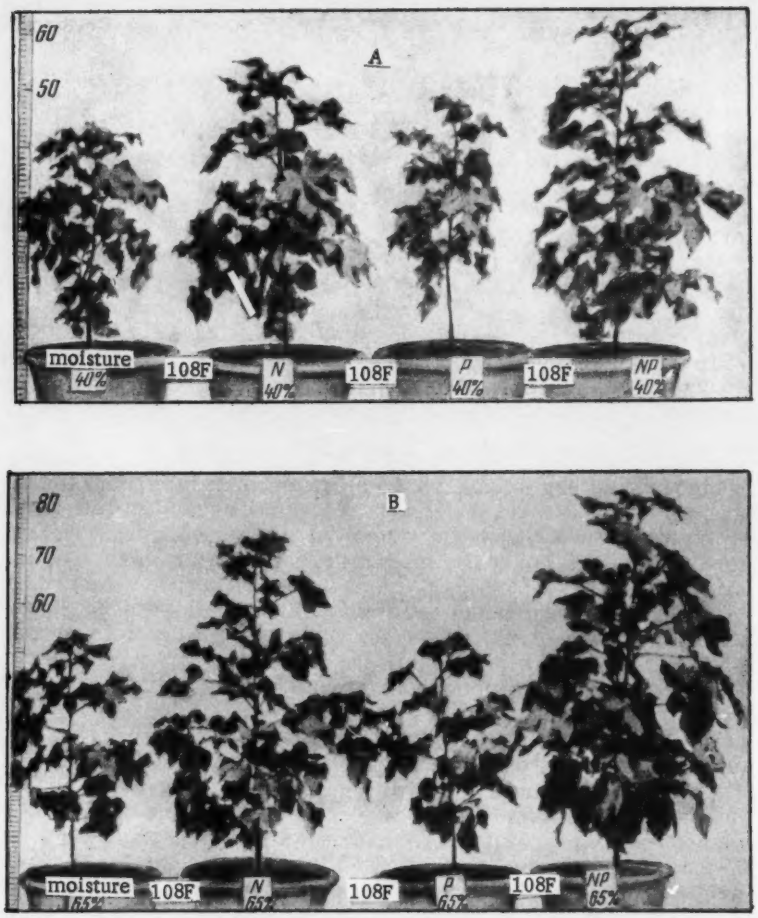

Fig. 3. Effect of soil moisture and mineral nutrition on growth and development of cotton plants, variety 108-F.
A) At 40% soil moisture; B) at 65% soil moisture.

more effective in their action than phosphorus alone, while at the stage of flower-bud formation, the greatest amount of bound water was observed, on the contrary, in the treatments given supplementary feeding of phosphorus alone (Fig. 1).

It can also be seen that a larger amount of total and free water and a smaller amount of bound water was observed, as usual, in the leaves of plants grown at 65% of field capacity, as compared with plants grown at 40% of field capacity.

A decrease in free water and an increase in bound water with an insufficient water supply has been noted by many other authors [2, 3, 19-31], which, with the high osmotic pressure of the cell sap, leads to a decrease in yield [3].

With optimum soil moisture available to plants throughout the growing period, an average amount of free water occurs [32], and there is a definite optimal ratio of free to bound water [23-25, 33-36].

Analysis of the data of our experiment, as the ratio of free to bound water in leaves of the cotton plant, at two values of soil moisture during the process of growth, supports a similar interrelationship (Table 1).

From the data given in Table 1, an increased ratio of free to bound water can be seen in leaves of plants grown with optimal soil moisture (65%), as compared to plants grown with insufficient moisture (40%), sufficient water being one of the necessary conditions for the normal living activity and growth of plants.

We have determined the amount of colloidal- and osmotically bound water in leaves of the cotton plant. The data on the amount of osmotically bound water are given in Table 2.

Here it should be noted that, in general, almost all bound water in the leaves of cotton plant is represented by osmotically bound water.

From the data of Table 2, one may conclude that the mineral supplementary feedings, particularly phosphorus combined with nitrogen, to a certain degree increased the amount of osmotically bound water, which is explained by an increase in osmotic pressure and in cell sap concentration under these conditions.

Along with this, we note a general increase in osmotically bound water in leaves of cotton plants of all experimental treatments given insufficient water supply (40%) as compared with optimal water supply (65%). The increase in osmotically bound water observed with the insufficient water supply is a consequence of the increase in osmotic pressure and in suction tension, which increase not only because of a decrease in the hydration of plant tissues, but also because of a breakdown of complex organic substances [22].

An increase in total bound water in a period of insufficient soil moisture may take place, also, as a result of increase in hydrophylic colloids and of quantitative changes in protoplasmic proteins [37].

Now let us turn to the data on the change in osmotic pressure and cell sap concentration, given in Table3.

The data of Table 3 show that a relatively high osmotic pressure and high cell sap concentration are observed throughout the whole growth period in the leaves of cotton plants which have received mineral fertilizer, particularly nitrogen and nitrogen plus phosphorus, which agrees with the conclusions of other investigators [2, 4, 39].

It is altogether natural that osmotic pressure and cell sap concentration were significantly lower in plants grown with optimal soil moisture (65%) than in plants grown with insufficient moisture (40%), this difference determining the high productivity of plants of the first treatment [29, 39, 35, 38-41].

We have determined the content of various forms of nitrogen (total, protein, and nonprotein), as well as forms of phosphorus in the leaves of cotton plants. The data obtained are given in Table 4.

From the data of Table 4, it follows that the amount of all forms of nitrogen in cotton leaves was greater in the treatments with nitrogen, and particularly nitrogen-phosphorus, than in the controls or in plants which received only phosphorus. An increased amount of nonprotein nitrogen was observed only in plants given nitrogen alone. The amount of protein nitrogen was, on the contrary, greater in the leaves of cotton plants with nitrogen-phosphorus supplementary feedings. If the content of protein nitrogen in leaves given the latter treatment (NP) compared with the amount of bond water in the same plants, it is not difficult to see the positive correlation between these two values [42]. This relationship is quite reasonable, since proteins, which are hydrophylic colloids, are capable of retaining a large amount of water. The increased hydrophylic property of colloids, as was already noted, contributes to a high rate of physiological processes, an increase in resistance to unfavorable factors [43], and an assurance of high productivity of the plants in general.

It is particularly because of this that the maximal yield of cotton fiber was obtained in the treatment with nitrogen-phosphorus supplementary feeding, on the background of optimal soil moisture, consisting of 133 g of fiber per plant at 65% soil moisture, and 90.6 g at 40% soil moisture, against a corresponding 56.2 g and 46.6 g with nitrogen feeding along, 24.18 g and 20.46 g with phosphorus feeding alone, and 24.79 and 20.3 g in the control, nonfertilized treatment.

By comparison of the data on the phosphorus content of cotton plant leaves with the content of bound water in the same treatment at various levels of soil moisture, we may conclude that no significant increase in the total bound water content is observed with an increase in inorganic phosphorus. From this, one may suppose that the increase in the content of inorganic phosphorus forms, as poorly hydrophylic compounds, does not play a large part in the composition of osmotically active substances in the cell sap [2, 4].

Let us now consider the data on the transpiration rate, given in Fig. 2.

From these curves, we see that the smallest transpiration rate of cotton plants was seen in the plants best supplied with nutrient substances, i.e. in the treatment given nitrogen and phosphorus feeding, while it was higher in the plants given phosphorus treatment alone, or not fertilized at all.

The lowering of transpiration rate in the first case is explained by increased hydrophilic properties of colloids and by a higher content of bound water in the leaves of plants given nitrogen and phosphorus fertilizers, which completely agrees with the conclusions of other investigators [2-4, 9-13, 42].

Even though the maximal total expenditure of water for transpiration (232 kg per vessel) throughout the whole period of growth was noted in the treatment given both nitrogen and phosphorus, in the plants most highly developed (Fig. 3), the productive transpiration there was higher in spite of this. The more economic, rational expenditure of water in plants of a given treatment was related to their high yield.

The differences in transpiration rate in the course of ontogenetic development — an increase prior to flower-bud formation, in which stage it reaches the highest limit, then a gradual decrease, with a short increase during the period of maturation (August 27) is explained by a dissimilar transpiring surface and internal qualitative changes, related to the redistribution of various forms of water, and also to increase or decrease in the degree of colloid hydration.

SUMMARY

1. Mineral nitrogen and phosphorus fertilizers have a great effect on the fractional composition of water in the leaves of the cotton plant. Thus, the phosphorus fertilizers at an earlier period (flower-bud formation) lower the amount of free water, as compared to nitrogen or nitrogen + phosphorus treatments. At the same time, at the flowering stage, or at a later stage, they, on the contrary, somewhat increase the amount of this water fraction.

Under the same conditions, the reverse relationship is observed in the content of mainly osmotically bound water; a preponderance of this fraction during flower-bud formation in the treatments given phosphorus, and a significant increase in the later stages in the treatments given nitrogen, and particularly nitrogen plus phosphorus feeding.

2. Soil moisture also affects the redistribution of various forms of water during growth. In the leaves of plants grown at optimal soil moisture (65%), as compared with the plants at insufficient soil moisture (40%), there was, throughout the whole growth period, an increased ratio of free to bound water, which is one of the necessary conditions for the normal life and growth of plants.

3. Nitrogen, and particularly nitrogen plus phosphorus fertilizers (N + P) considerably increased the osmotic pressure and cell sap concentration in the leaves of cotton plants. However, in plants grown at optimal soil moisture (65%) with application of the same fertilizers, the values of these indicators were lower than in plants grown at insufficient soil moisture (40%), which has determined the high productivity of the first treatment.

4. Nitrogen fertilizers in combination with phosphorous increased the amount of protein nitrogen in the leaves of cotton plants, which leads to an increase in hydrophilic colloids. Simultaneously, these fertilizers considerably increased the amount of total bound water, all of which, taken together, contributes to a higher rate of the physiological processes which ensure high productivity and yield of the plant as a whole.

5. An increase in the content of inorganic forms of phosphorus, which are poorly hydrophylic compounds, does not play a large part in the composition of the osmotically active substances of the cell sap. Because of this, no increase of the bound water content is observed with an increase in inorganic phosphorus.

6. The decrease in transpiration rate of cotton plants in the treatment given nitrogen and phosphorus feeding is explained by a highly hydrophilic state of colloids and by a high content of bound water in the plant leaves. More economic expenditure of water in plants of a given treatment was connected with their high yield.

7. Further studies of the nature and degree of changes in the indicators of the water regime and of their interrelationship to the other physiological processes will permit planning the more rational use of fertilizers for increase in yield of cotton plants.

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HORMONAL FACTORS IN THE FLOWERING OF PLANTS

M. Kh. Chailakhian

K. A. Timiriazev Institute of Plant Physiology, USSR Academy of Sciences, Moscow

1. Introduction

About twenty years ago we proposed [1] a concept concerning the presence in plants of certain hormonal substances of high physiological activity, or flowering hormones; the name florigen was applied to this group of substances, until such time as the individual substances might be distinguished. This concept was based on experiments which showed that reproductive development of plants, and flowering in particular, is conditioned by external and internal factors other than their vegetative growth; on experiments in which the effect of a differentiated light regime was used on separate parts and organs of plants, leading to a discovery of a mechanism of photoperiodic reaction — the leaves, as receptive organs, interacting with other organs with respect to the nature, direction, and paths of translocation of substances necessary for flowering from leaves into stem buds; and also on experiments with grafts, in which vegetating components began to flower under unfavorable conditions of day length because of substances coming from the leaves of the flowering grafted components. Then, it was proposed that the flowering hormones (florigen) have a similar nature in long-day, short-day, and neutral types. This hypothesis was based on experiments with grafts, in which short-day species flowered under long-day conditions because of the substances produced in the leaves of the stocks of a long-day species — Jerusalem artichoke on sunflower, and Maryland Mammoth tobacco on Samson tobacco [2, 3]. Soon, it was shown that the seedlings of a biennial long-day species, henbane, flowered on short day-length when a short-day variety of tobacco, Maryland Mammoth, was grafted to them, because of the substances of that species; while the plants of Maryland Mammoth tobacco flower under long-day conditions as a result of grafting to them the leaves of long-day species — henbane and Nicotiana silvestris [4, 5]. In further experiments, it was completely demonstrated that short-day and long-day species would flower under conditions of unfavorable day-length when reciprocally grafted, because of the substances flowing from the flowering components of the graft [6, 7].

Investigations by a large number of authors have shown that the substances conditioning flowering of plants are not the usual metabolites of plants — carbohydrates, or nitrogeneous substances [1, 8-10]. Equally, it has been shown that these substances are not growth hormones or auxins, since the content of the latter is always greater under long-day conditions, when a more intensive growth of plants is taking place, as well as the flowering of long-day, but not of short-day, species [11]. Even though Overbeek [12] has succeeded in considerably speeding up flowering and fruiting of pineapples, and Kholodnii and Kocherzhenko [13] succeeded in causing flowering and fruiting of lemon shoots, by means of weak aqueous solutions of synthetic growth substances, alpha-naphthalenacetic and 2, 3 — dichlorophenoxyacetic acids, all the subsequent experiments with many other annual and perennial plants did not give successful results, and it became clear that these substances could not be regarded as direct regulators of the processes of flowering.

The question of the nature of flowering hormones remained unsolved for a long time, but very recently a great deal of interest in this question has again appeared; at the last meeting of the American Society of Plant Physiologists and the Physiological Section of the Botanical Society of America, a report by Bonner [14] on the topic "State of the Florigen Problem" was presented and discussed. The reason for the new interest in the problem of florigen, i.e. in the hormonal factors of flowering, is undoubtedly the discovery of the gibberellins.

2. Effect of Gibberellins on Growth and Flowering of Plants

Gibberellins were discovered in secretions of the fungus Gibberella fujikuroi, a parasite of rice, and turned out to be substances of high physiological activity, active in very high dilutions. The discovery of gibberellins was followed by widespread testing of their effect on growth and development of plants, as well as on physiological manifestations and morphological processes [15, 16].

The investigations on the effect of gibberellins on the growth and flowering of plants are of particular interest. From the beginning, it was established that the gibberellins had a sharply expressed stimulating effect on the growth of seedlings of many annual and perennial plants [17-20], and a particularly strong reaction was shown in dwarf forms of such plants as pea, corn, etc. [21, 22].

Somewhat later, it was shown that the gibberellins have a similar great effect on flowering [20, 23-32]. These investigations are distinguished by complete coincidence of the results obtained, in spite of the variation of the species tested and of differences in the conditions of the experiments.

When treated with gibberellins, all the following species formed stems, flower-buds, and flowers under short-day conditions: Nicotiana silvestris, Rudbeckia (Rudbeckia bicolor and R. hirta), annual henbane (Hyoscyamus niger), decorative bean (Phaseolus multiflorus), Samolus parviflorus, Crepis tectorum and C. leontodoides, catchfly (Silene armoria), nipplewort (Lapsana communis), Bryophyllum (Bryophyllum crenatum and B. diagremontianum), Adonis flammeus, Arabidopsis, petunia, dill, radish, lettuce, spinach, and some others.

When treated with gibberellin, seedlings of all of the following winter forms, biennials, and herbaceous perennial, began to form stems, and then flowered and bore fruit, under long-day conditions and without exposure to lowered vernalizing temperatures: winter rape, winter endive, winter variety of Arabidopsis thaliana, Centaurium minus, biennial henbane, carrot, parsley, cabbage, turnip (Brassica rapa,) beet, turnip (Brassica napus rapifera), and goldenrod (Solidago virgo aurea).

All of the following short-day species treated with gibberellins failed to form flower-buds and did not flower under long-day conditions, but remained in a state of vegetative growth; cocklebur (Xanthium saccharatum), Biloxi and Harbin soybean (Soja hispida), Kalanchoe (Kalanchoe blossfeldiana), Mammoth tobacco (Nicotiana tabacum), red Perilla (Perilla nankinensis) and millet (Panicum miliaceum).

From this, one may conclude with complete certainty that gibberellins cause flowering of long-day species under short-day conditions by substituting for the photoperiodic induction of the long day, and cause flowering of the seedlings of winter forms and biennials in the first year of their life, substituting for vernalization; but they cannot induce flowering of short-day species under long-day conditions.

This became completely clear in our experiments [3], in which long-day and short-day species, as well as one winter variety, were studied in parallel. Under short-day conditions, treated with gibberellin, Nicotiana silvestris and Rudbeckia formed stems, flowers, and fruits; decorative bean flowered, and oats, variety pobeda, formed stems and differentiated heads; while control plants remained in the stage of rosette and vegetative growth, or, as in oats, had less developed heads (Fig. 1).

When treated with gibberellin, plants of winter rape and a first-year perennial, goldenrod, formed stems, flowered, and bore fruit, under long-day conditions, and the stems elongated without vernalization under short-day conditions, while the control plants remained at the rosette stage (Fig. 2).

At the same time, under long-day conditions the short-day species Mammoth tobacco and red Perilla considerably increased their growth due to the action of gibberellin, but remained at the stage of vegetative growth, while Harbin soybean and Japanese millet did not flower any sooner, but began reproductive development at the same time as control plants.

Thus, the gibberellins turned out to be substances which actively affect the reproductive development of long-day spring and winter varieties and do not affect the reproductive development of short-day species. The sharply expressed difference in action of gibberellins on plants of different photoperiodic groups immediately became clear.

Comparison of these data on the action of gibberellins on plants with the results obtained in experiments with grafts show that the gibberellins are not flowering hormones, which are of the same nature for both long-day

Fig. 1. Effect of gibberellin on growth and development of Rudbeckia under short-day conditions.
At the left) two plants which received 300 ug gibberellin each, flowering; at the right) control plants, which are at the rosette stage; 56 days after the beginning of the experiment (photographed August 28, 1957).

and short-day varieties, and that the important problem is the clarification of the interrelationship between the gibberellins and florigen.

3. Effect of Gibberellins on Formation and Growth of Stems

To solve the question of the interrelationship between florigen and the gibberellins, we have carried out experiments on the effect of gibberellin on plants, and also carried out a general critical analysis of the experimental data already available, our own, as well as that of other authors.

During the growing season of 1957, with the participation of L. P. Khlopenkova and T. N. Konstantiva, at the Plant Physiology Institute of the USSR Academy of Sciences, we carried out experiments with long-day species, Nicotiana silvestris and Rudbeckia (Rudbeckia bicolor), and short-day species, Mammoth tobacco (Nicotiana tabacum) and red Perilla (Perilla nankinensis).

The seeds of tobacco, Rudbeckia and Perilla were planted in flats — Perilla on March 4, Rudbeckia on March 12, and tobacco on March 26; after a single thinning of the seedlings, the young plants were transplanted into 20-centimeter clay pots — Perilla on April 9, Rudbeckia on May 25, and tobacco on June 1 and June 29. From the time of emergence until the beginning of the experiment, the plants of the long-day species were placed under conditions of a short, 9-hour day, which in the greenhouse were created by wheeling the plants

Fig. 2. Effect of gibberellin on growth and development of winter rape under long-day conditions, and without lowered-temperature treatment.
At the left) two plants which received 1800 ug gibberellin each, flowering; at the right) control plants, which are at a rosette stage; 80 days after the beginning of the experiment (photographed September 21, 1957).

TABLE 1

Effect of Gibberellin on Growth and Formation of Flower Organs Under Different Day Length Conditions (experiment began July 3, 1957)

Plant	µg gib-berellin	Plant height, cm				Formation of flower organs (flower-bud formation)	
		Beg. of exp., July 4		34 days later, 8/6			
		L	S	L	S	L	S
Long-day species							
Nicotiana silvestris	300	Rosette	Rosette	36	30	13.VIII	12.X*
	200	»	»	30	19	13.VIII	18.X*
	—	»	»	13	Rosette	16.VIII	None
Rudbeckia	300	Rosette	Rosette		51	22.VII	1.VIII
	150	»	»	106	42	22.VII	4.VII
	—	»	»	93	Rosette	22.VII	None
Short-day species							
Mammoth tobacco	300	3	3	76	67	*	19.VIII
	200	2	3	63	53	»	21.VIII
	—	2	3	20	22	»	16.VIII
Red Perilla	300	39	39	111	103		20.VII
	200	41	38	107	99	»	21.VII
	—	39	40	76	82	»	21.VII

* Plants of Nicotiana silvestris on short-day, and of Mammoth tobacco on long-day, were given an additional amount of gibberellin, after August 2.

Fig. 3. Growth and flowering of Nicotiana silvestris, treated with gibberellin under long-day conditions. Plants received 300 μg (on the left) and 200 μg (in the middle) of gibberellin; control plants (on the right). All plants flowered 56 days after the beginning of the experiment (photographed August 28, 1957).

Fig. 4. Growth of Nicotiana silvestris treated with gibberellin under short-day conditions. Plants received 600 μg (on the left) and 350 μg (in the middle) of gibberellin — after stem formation, plants went into flowering on October 12 and October 18; control plants (on the right) are at the rosette stage; 80 days after the beginning of the experiment (photographed September 21, 1957).

Fig. 5. Growth and flowering of Mammoth tobacco treated with gibberellin under short-day conditions. Plants received 300 μg (on the left) and 200 μg (in the middle) of gibberellin, and are forming flower-buds; control plants (on the right) are beginning to flower; 56 days after the beginning of the experiment (photographed August 28, 1957).

into a dark photoperiodic house from 6 P.M. to 9 A.M.; the plants of the short-day species were on a long natural day with supplementary electric light from the middle of April to the beginning of August.

At the beginning of the experiments, plants were placed under different day-length conditions, and treatment with gibberellins was started. Gibberellin A 3 and gibberellic acid (Eli Lilly and Company), obtained from Professor A. Lang (Los Angeles) were used, in a concentration of 0.02%; they were applied daily to the plants by drops, a drop containing approximately 10 μg of substance; control plants were treated with drops of water. Altogether, three experiments were carried out.

In the first experiment, the question was raised as to how gibberellin acts on the formation and growth of stems of long-day and short-day species under different day lengths, and whether there is any connection between the growth of stems and formation of flower organs. The experiment was started on July 3, with all the above-mentioned species: one-half of the plants were placed on long-day (L), the remainder were left on a short, 9-hour day (S), and treatment with gibberellins was begun. Daily, one drop of gibberellin solution was placed into the central part of a rosette plant or on the tip of a stemmed plant. The plan of the experiment consisted of three treatments: 1) plants received 300 μg of gibberellin for 30 days, 2) 200 μg gibberellin for 20 days (in the case of Rudbeckia 150 μg), 3) control plants. The experiments were carried out in triplicate. Throughout the experiment, phenological observations were made and the growth of the plants was measured.

The results of the experiment are given in Table 1 and in Figs. 3-10.

The data of Table 1 show that the long-day species kept under short-day conditions, which are unfavorable for their flowering, are not capable of forming stems, but are left at the rosette stage, while the short-day species kept under long-day conditions, unfavorable for their flowering, are capable of forming stems, just as under short-day conditions.

Fig. 6. Growth of Mammoth tobacco treated with gibberellin under long-day conditions. Plants received 600 μg (on the left) and 350 μg (in the middle) of gibberellin; control plants (on the right). All plants are in the vegetative stage; 80 days after the beginning of the experiment (photographed September 21, 1957).

The same data, as well as Figs. 3-6, show that, in both species of tobacco, gibberellin acts as a stimulator for the growth of stems, both on long and on short-day. In the long-day Nicotiana silvestris, exposed to long-day, the growth of stems takes place more intensively in plants treated with gibberellin, and flower-buds are formed earlier than in control plants; on short-day they show a qualitative difference, as distinguished from control plants, which remain at the rosette stage; the plants treated with gibberellin form stems, and consequently pass on to formation of flower organs. In short-day Mammoth tobacco, exposed to short-day, the stem growth is increased under the treatment with gibberellin, while flower-bud formation is somewhat retarded; on long-day, the stem growth is also increased by gibberellin treatment, but flower-bud formation and flowering do not take place even after application of a large amount of gibberellin.

A similar picture is observed when the behavior of a long-day species, Rudbeckia, and a short-day species, red Perilla, are compared (Figs. 7-10). In Rudbeckia, stem growth on long-day is somewhat increased as a result of gibberellin treatment, and the plants form flower buds in the same way as control plants; on short-day, again a qualitative difference is shown — the control plants are left at the rosette stage, while the experimental plants form stems and flower. In red Perilla, under both long-day and short-day conditions, stem growth is increased after gibberellin treatment, but no flowering occurs on the long-day.

The results of the experiment show that in the long-day species the formation and growth of stems is closely connected with the formation of flower organs, and must necessarily precede flowering, while in the short-day species this connection is not observed, since they can form stems both on short-and on long-day. In addition, the present experiment has shown that in the long-day species the formation of flower organs and flowering takes place in the same way on long and on short day, provided that stem growth takes place; in short-day species, stem growth does not predetermine the formation of flower organs, and they do not flower on long day.

In the second experiment, the question was posed as to whether gibberellin acts directly on the processes which take place in the meristematic tissues of stem apices, or whether it undergoes a transformation in the leaves, and only then is translocated into the tissues of stem apices.

For this purpose, four-month-old rosette plants of Rudbeckia and vegetative plants of Perilla were treated in such a way that all leaves on each plant were removed except one well-developed leaf, and all lateral shoots were removed, leaving the central apical shoot; later, all newly formed shoots and leaves were cut off daily. The experiment with such living models was begun on July 3, when half of the plants were placed on a long-day and the other half, on short-day.

On the same day, the gibberellin treatment was started by applying one drop to each plant daily. The experiment consisted of three treatments, each in duplicate: 1) a drop of gibberellin was applied to a central part

Fig. 7. Growth and development of Rudbeckia under the influence of gibberellin, under long-day conditions. On the left) plant which received 150 μg gibberellin, on the right) control plant. Both plants form flower-buds; 23 days after the beginning of experiment (photographed July 26, 1957).

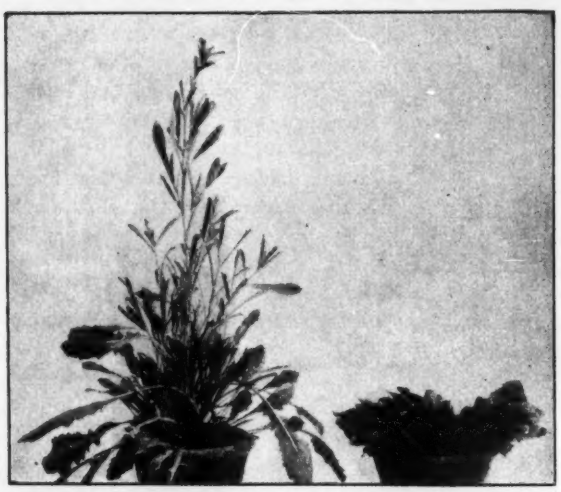

Fig. 8. Growth and development of Rudbeckia under the influence of gibberellin, under short-day conditions. On the left) plant which received 150 μg gibberellin, and formed a stem; on the right) control plant at the rosette stage; 23 days after the beginning of the experiment (photographed July 26, 1957).

of the rosette, and later to the stem apex, of Rudbeckia and to the stem apex of Perilla; 2) a drop of gibberellin was placed on the middle part of the leaf blade; 3) control, a drop of water was placed on the stem apex and on the leaf. Treatment was carried out for 20 days, so that every plant received approximately 200 μg of gibberellin.

The results of this experiment are given in Table 2 and Figs. 11 and 12.

The data of Table 2 show that in Perilla the introduction of gibberellin both through the stem apex and through the leaf did not affect the reproductive development of plants; on long-day they remained in the stage of vegetative growth, on short-day they began to flower. However, the effect on stem growth was shown quite definitely; under both long and short-day conditions the stem growth was much more intensive when gibberellin was introduced through the stem apex than when it was introduced through the leaf, and in the latter treatment it was more intensive than in the control (Fig. 11).

In Rudbeckia, on long-day, the stem height was greater, and flower-bud formation and flowering occurred earlier after the application of gibberellin drops to the stem apex than in the treatment in which gibberellin was introduced through the leaf. Similarly, on short-day, the stem height was greater and flower-bud formation occurred earlier when gibberellin was introduced through the stem apex; in the treatment in which the gibberellin was introduced through the leaf, there was no flowering, and in the control, stems were not formed and plants remained at the rosette stage (Fig. 12).

The experiment with the living models of Rudbeckia and Perilla has shown that growth and flowering takes place more intensively with direct action of gibberellin on the stem apex than in the case of action through the leaf; that, consequently, gibberellin is not subjected to any transformation in the leaf on long-and short-day, but directly affects the processes taking place in the tissues of the stem apex; the weakening of the effect, when it is introduced through the leaf, is connected with the distribution of gibberllin in the leaf blade during its translocation to the stem apex.

In addition, the experiment described has shown that flower formation in Rudbeckia is always preceded by formation and growth of the

Fig. 9. Growth and development of red Perilla under the influence of gibberellin, under short-day conditions. On the left) Plant which received 200μg gibberellin; on the right) control plant. Both plants flowered; 40 days after the beginning of the experiment (photographed August 13, 1957).

Fig. 10. Growth of Perilla under the influence of gibberellin under long-day conditions. On the left) Plant which received 200μg gibberellin; on the right) control plant. Both plants are in a vegetative state; 40 days after beginning of the experiment (photographed August 13, 1957).

stem, and there is a general direct correlation between the rate of stem growth and the rate of flower-bud and flower formation under both long- and short-day conditions; that in Perilla, under short-day conditions, there is no similar relationship between the rate of stem growth and the rate of flower-bud and flower formation.

In the third experiment, the question was raised whether or not leaves placed on a day-length unfavorable for flowering exert an inhibiting action on the effect of gibberellin.

The experiment was set up on August 5, with Rudbeckia, under short-day conditions, in duplicate; the plants were treated with a gibberellin solution of the same concentration as in the first two experiments, by means of daily application of one drop to the central part of the rosette, and later to the plant apex. The treatment was continued for 15 days; in some plants all the leaves, except the smallest ones, were removed, while in others, control plants, the leaves were left intact.

The results of this experiment, terminated on December 17, are given in Table 3.

From Table 3, it can be seen that rudbeckia plants treated with gibberellin under short-day conditions began to form flower-buds in approximately two months, and flowered towards the end of the third month. The plants formed flower-buds and flowered somewhat earlier and were heavier in those cases where leaves were left on the plant. When leaves were removed, stem elongation took place more intensively, but the plants as a whole were weaker.

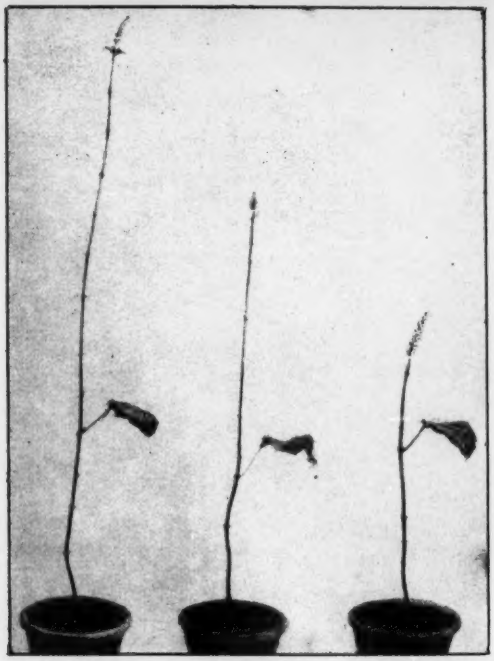

Fig. 11. The effect of the method of gibberellin application on growth of stems and flowering of red Perilla under short-day conditions. The gibberellin solution was placed on the tip of the stem (at the left), on the leaf (in the middle); control plant (on the right). All plants flowered; 23 days after the beginning of the experiment (photographed July 26, 1957).

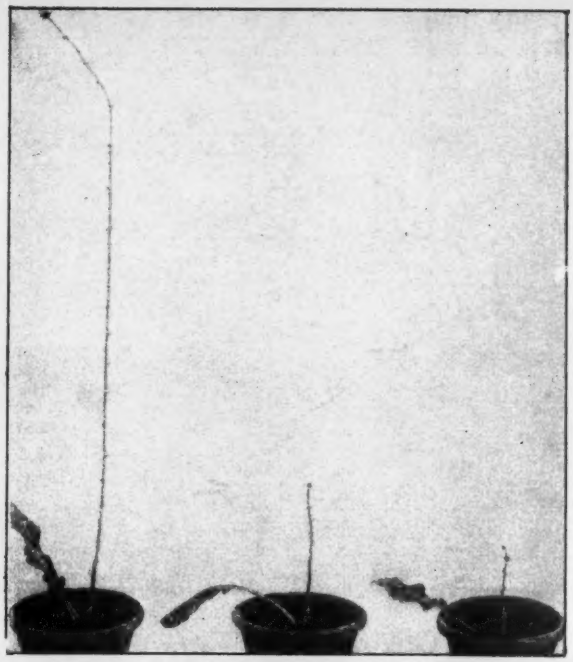

Fig. 12. The effect of the method of gibberellin application on stem growth of Rudbeckia under long-day conditions. Gibberellin solution was applied to the stem apex (on the left), to the leaf (in the middle); control plant (on the right). All plants flowered; 23 days after the beginning of the experiment (photographed July 26, 1957).

TABLE 2

Effect of Gibberellin on Growth and Development of Plants when Introduced Through Stem Apex and Through the Leaf (experiment started on July 3, 1957)

Experimental treatment	Day length	Amount of gibberellin in µg	Flower-bud formation	Flowering	Stage of development at end of experiment	Stem ht. from leaves up to stem apex, cm	The end of the experiment
Rudbeckia							
Stem apex	11	200	20.VII	5.VIII	Flowering	98	19.VIII
Leaf	11	200	25.VII	10.VIII	»	34*	
Control	11	—	25.VII	10.VIII	»	42	
Stem apex	20	200	30.VII	18.VIII	Flowering	50	26.IX
Leaf	20	200	19.VIII	—	»	14	
Control	20	—	—	—	Rosette	—	
Perilla							
Stem apex	11	200	—	—	Vegetative growth	55	26.IX
Leaf	11	200	—	—	» »	28	
Control	11	200	—	—	» »	18	
Stem apex	20	200	22.VII	5.VIII	Flowering	62	19.VIII
Leaf	20	200	23.VII	7.VIII	»	38	
Control	20	—	24.VII	6.VIII	»	21	

* Leaves fell earlier than in control.

TABLE 3

The Effect of 150 μg of Gibberellin on Growth and Development of Rudbeckia Plants With and Without Leaves (experiment started August 5, 1957)

Experimental treatment	Flower-bud formation	Flowering	Stage of develop. at end of experiment	Plant height from base to tip in cm	Wet weight per plant in g
With leaves	1.X	25.X	Fruit-bearing	73	80
»	—	—	Rosette	—	32
Without leaves	3.X	28.X	Fruit-bearing	108	50
»	—	—	Rosette	—	18

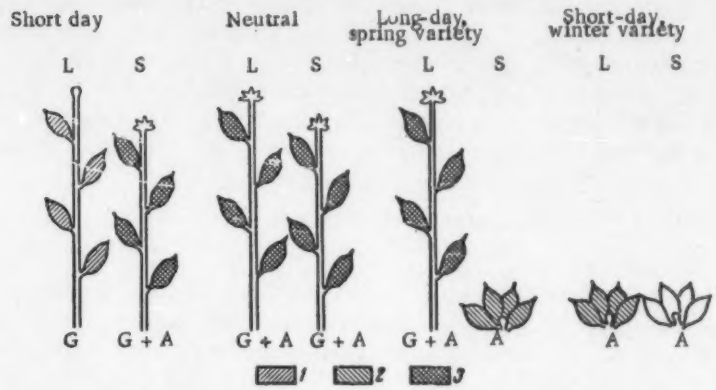

Fig. 13. Scheme for formation of flowering hormones in different plant species.

This experiment makes it possible to conclude that rudbeckia leaves placed on a short day, unfavorable for flowering, not only do not have an inhibiting action on the gibberellin effect, but even somewhat stimulate flowering, and considerably increase the accumulation of organic substance by plants; along with this, these leaves show a retarding effect on growth and elongation of plant stems.

4. Gibberellins and Anthesins as Hormonal Factors in Stem and Flower Formation

The experimental results indicate that it is doubtful whether the gibberellins should be considered as substances which in long-day species and winter varieties induce a chain of reactions leading to the formation of florigen, as the sum of flowering hormones [31]. A more probable hypothesis is that they enter into the composition of flowering hormones of a general nature, as a group of substances which are lacking in long-day species under short-day conditions, and in winter varieties and biennial seedlings without action of lowered temperatures. Since, under short-day conditions and without vernalization, plants of these species are in the rosette stage, or have very shortened stems, then it must be admitted that gibberellins are substances which influence mainly, and perhaps exclusively, the formation and growth of stems, and that in the spring long-day varieties this formation takes place faster than in nonvernalized winter varieties and biennials.

The capacity of long-day species, when treated with gibberellins, not only to form stems, but also to flower under short-day conditions indicates that substances are formed in their leaves which are necessary for the formation of flower organs, not only under long-day conditions, but also under short-day conditions. In rosette and shrub varieties the effect of these substances is not visible at all, and in species with a slowly growing, shortened stem it appears very late, since flowers do not arise from leaves, but they always appear from buds initiated on the growing stems and shoots. Winter forms and biennial seedlings in the first year of life, which have not been vernalized, form stems, flower, and bear fruit under the influence of gibberellin on long-day,

while on short-day, only formation and growth of stems occurs. This indicates that under long-day conditions they contain substances necessary for formation of flower organs, but they lack gibberellins; under short-day conditions, however, they have neither the substances necessary for formation of flower organs, nor gibberellins.

All short-day varieties, both on short and on long day, always form stems, and consequently do not experience a lack of gibberellins, as substances which affect the formation of stems. Under long-day conditions, they do not flower when treated with gibberellins, while under short-day conditions their flowering takes place approximately at the same time as flowering of control plants. Under long-day conditions, the short-day species lack that group of substances which act directly on flower formation, and which cannot be replaced by gibberellins.

Neutral varieties, which are primitive and not specialized, are capable of forming stems and flower organs under both long-and short-day conditions, and they have a sufficient amount both of gibberellins and of the substances which are necessary for formation of flower organs.

Thus, the flowering hormones, or florigen, general for all plant species, encompass both gibberellins and substances necessary for formation of flower organs. In his time, Kholodnii [33], while criticizing the term "florigen" which we proposed, suggested the term "anthesin", for designating the hormone affecting formation of flowers. One may boldy accept this term, not, however, as a substitute for "florigen", but for designating that group of substances which is necessary for formation of flower organs, and which enter into the composition of florigen. To say it in fewer words, one may suppose that florigen consists of two groups of substances — gibberellins and anthesins.

The interrelationship of gibberellins and anthesins in neutral, short-day and long-day varieties, both spring and winter types, is represented in schematic form in Fig. 13.

The scheme in Fig. 13 shows that flowering of neutral, short-day and long-day species takes place in those cases in which two groups of substances — gibberellins and anthesins, which together compose florigen (flowering hormones, general for all species), are translocated from plant leaves into the stem buds. Absence of flowering of long-day species on short-day is caused by lack of gibberellins; absence of flowering of short-day varieties on long-day is caused by the lack of anthesins. Absence of flowering in winter and biennial varieties, which were not exposed to the effect of lowered temperatures, is caused by the absence of gibberellins on long-day, and by the absence of gibberellins and anthesins on short-day.

The present explanation is based on those new facts which were obtained as a result of studying the effect of gibberellins on growth and development of plants, and from comparison of results obtained with the data of graft experiments between short-day and long-day spring and winter varieties, as well as seedlings of biennial forms. This explanation, of course, needs further experimental and theoretical support.

However, at the present time some data are already available, which may be brought to support the proposed hypothesis. Thus, in the early graft experiments of Melchers[4] it was shown that nonvernalized one-year old seedlings of biennial henbane (Hyoscyamus niger) form flower primordia due to the action of grafted leaves of short-day Maryland Mammoth tobacco, placed under conditions of both short-and long-day; on short-day, the grafts themselves formed flower primordia, while on long-day, they remained in the vegetative state. On the basis of this, Melchers proposed a hypothesis concerning the presence in all annual spring varieties of another hormone, besides florigen, which is formed according to the day length, a vernaline, which in winter and biennial plants is formed in the process of vernalization. In the subsequent experiments of Melchers and Lang [5], it was established that, in contrast to the tobacco, grafted leaves of the long-day annual henbane induced formation of flower primordia in the stock rosettes of biennial henbane, only in those cases when they were kept under long-day, but not under short-day, conditions; in connection with this experiment, the authors expressed doubts concerning the vernaline hypothesis. Quite recently, Sarkar [32] found a lack of correlation between the action of vernalizing lowered temperatures and the action of gibberellic acid on seeds and on young and old rosettes of the winter species Arabidopsis thaliana, and concluded that gibberellin is not identical to vernaline — the hypothetical end product of vernalization. The contradictory results of the experiments of Melchers and Lang can be explained by the proposed scheme, since in the case of tobacco, the gibberellins, which are lacking in the rosettes of biennial henbane, are present both on long-and on short-day; while in the case of the annual henbane, they are present only on long day.

In our experiments [34] with grafts of winter rape onto spring varieties, rape and Abyssinian cabbage, it has been shown that the flowering of the winter grafts takes place only when the stocks are placed under long-day conditions, i.e. when they have gibberellins; with a direct action of gibberellins on the winter varieties, flowering also occurs only under long-day conditions, while under short-day conditions, only stem formation takes place. These coinciding results are also reflected in the proposed scheme.

In our other experiments [35], it has become clear that, with the presence of leaves which are placed under conditions of favorable day length, the inhibiting action of long-day leaves in short-day varieties, red Perilla, is considerably greater than the inhibiting action of short-day leaves in the long-day varieties — mustard, radish, and Rudbeckia. This difference is evidently connected with the fact that translocation into the stem apex of one group of substances, anthesins, is inhibited in Perilla while in Rudbeckia and other long-day varieties, that of the other group of substances, gibberellins, is retarded.

In the investigations of Stolwijk and Zeewaart [36] and Meijer [37], it has been established that when long-day species — henbane, petunia, Plantago major, Violaceae, and Arabidopsis thaliano, were kept on an 18-hour day of different spectral compositions, the plants formed stems and flowered fastest on blue, violet, and red-+ infrared light, while plants of the short-day species Salvia occidentalis flowered only on red and green light. It is possible that the formation of gibberellins favoring the growth of stems and flowering of the long-day species is predominant in blue and violet light, while in red light, the formation of anthesins favoring the flowering of the short-day species is predominant.

From the time of the discovery of photoperiodism [38], it was known that day-length has different effects on the processes of vegetative growth and of reproductive development, and, as our experiments [1] have shown, in long-day species the intensification and slowing of vegetative growth on long-day and short-day coincide with the speeding up or retardation of flowering; while in short-day species, the intensification of vegetative growth under long-day conditions takes place with retardation of flowering, and retardation of vegetative growth under short-day conditions occurs with the acceleration of flowering. Further, it has been established that, when plants are treated by auxins — heteroauxin and synthetic substances of similar activity, which increase the rate of growth processes, flowering of the long-day species is somewhat speeded up, while flowering of the short-day species is retarded [39-41]. It is probable that the auxins, which have a supplementary action on growth in the same direction as the gibberellins, favor the flowering of long-day species, but not short-day species, which do not suffer from the lack of gibberellins.

Finally, it has been established that the substances which favor the flowering of long-day spring varieties and vernalization of winter varieties are sugars [42-44], while the substances favoring the flowering of short-day species are nitrogenous substances [10, 45-47]. It may be supposed that the source for formation of gibberellins ($C_{19}H_{24}O_6$, $C_{19}H_{26}O_6$, $C_{19}H_{12}O_6$), which contain carbon, hydrogen, and oxygen, are sugars; while for formation of anthesins, nitrogenous compounds are needed, which makes probable the presence of nitrogen in the composition of anthesins.

5. Conclusion

The concept of flowering hormones, or florigen, has been based on those phenomena which have the general nature of photoperiodism in long-day and short-day species — the perception of a photoperiodic stimulus by leaves as receptive organs, translocation of the products of the altered plant metabolism from leaves into the stem buds, the presence of photoperiodic induction, flowering capacity in reciprocal grafts, dependence of the nature of the photoperiodic reaction on geographic origin and distribution of species, etc.

However, for the last twenty years many fundamental distinctions in photoperiodism of long-day and short-day species were established, to explain which a further development of our concepts concerning the hormonal nature of the process of plant ontogenesis is needed. Differences in the nature of the inhibiting action of leaves placed on an unfavorable day length, in relation to the spectral composition of light, in the interaction of the processes of vegetative growth and flowering, and in the reaction to the introduction of sugars and conditions of nitrogen nutrition, have already been discussed in the light of the concept of gibberellins and anthesins. It is most probable that further experimental and theoretical analysis will allow one to understand, in the light of this concept, even such important differences in the photoperiodism of long-day and short-day species as the reaction to the interruption of a long period of darkness by short periods of light, the reaction to supplementary light of

weak intensity, the relationship to temperature during light exposure and in darkness, the dependence of flowering on the products of metabolism supplied from the roots, and others.

The proposed explanation and the scheme of formation of flowering hormones in different plant species presupposes that the basis of such complex processes as growth and flowering of plants is the general process of plant metabolism, in which many compounds participate, particularly such substances of high physiological activity as auxins, vitamins, and enzymes [48, 49]. Particularly deserving of attention is the paper of Bouilienne [50], in which he suggests that florigen consists of several components, and proposes that these components may be, according to the data of Sironval [51], products of the hydrolytic activity of chlorophyllase, which were identified as vitamins E and K. Experiments we carried out [52] have shown that spraying plants with aqueous solutions of vitamins C, B_1 and P-P somewhat speeds up their flowering. On the other hand, new data were obtained recently, which show that under certain conditions some auxins and synthetic growth regulators affect the time of flowering of both long-day and short-day species (Laiboch and Kribben [53]; Salisbury [54, 55]; Liverman and Lang [56]; Hamner and Nanda [57]). All these data in general show that the auxins and some vitamins play an indirect role in the processes of flowering. The flowering hormones themselves should be regarded as products of plant metabolism which, arising under certain conditions, in turn affect the changes in plant metabolism which precede morphological processes. Insofar as the gibberellins are concerned, experimental data already are available which indicate their presence, as well as that of gibberellin-like substances, in the seedlings of fruits and seeds of many plant species [58-61]; with the help of extracts from the seeds of Echinocystis macrocarpa and kidney beans, the flowering of biennial henbane, Samolus parviflorus, and Bryophyllum crenatum was induced under conditions in which this could previously be done only with the aid of gibberellins [62-63].

The separation of flowering hormones or florigen into two groups of substances, gibberellins and anthesins, which is possible at present, does not change our general concepts [34, 64, 65] concerning the nature of the reactions basic to photoperiodism and vernalization, and concerning the adaptive significance of photoperiodism and vernalization, their relationship to the basic physiological processes, and the interaction of organs during flowering.

This separation, it seems to us, gives us a basis for understanding those distinctions which exist between neutral, short-day, long-day, spring and winter varieties, as well as a basis for prosecuting from all sides the investigation of plant ontogenesis, and, in particular, of the transformation of plants from vegetative growth to flowering and fruit-bearing.

SUMMARY

1. The concept of flowering hormones or florigen was based, among other factors, on the results of graft experiments, which proved that short-day and long-day species could be flowered under conditions of unfavorable day length by reciprocal grafts, through the activity of substances flowing from the flowering components of the graft. On the other hand, experiments on the effect of gibberellins on plants have shown that, while stimulating the growth of all plants, they accelerate flowering of seedlings of biennial, winter, and long-day species, but not that of short-day seedlings. Consequently, gibberellins are not the flowering hormones, which are of a similar nature for all plants.

2. The experiments described in this paper have shown that flowering of long-day species caused by the action of gibberellins is always preceded by formation and growth of stems, which does not occur in short-day species; that gibberellins are not transformed in the leaves under either long- or short-day conditions, but act directly on the processes taking place in stem buds; that leaves placed on unfavorable day length do not inhibit the effect of gibberellins on formation of stems and flowers. Consequently, the gibberellins are hormones necessary for stem formation.

3. A comparison of experimental data and their theoretical analysis permit one to conclude that flowering hormones of florigen consist of two groups of substances: gibberellins, necessary for formation of stems, and anthesins, necessary for formation of flowers. Neutral species have both gibberellins and anthesins on any day length; failure to flower in long-day species under short-day conditions is caused by the lack of gibberellins, while the short-day species fail to flower on long-day because of the lack of anthesins; nonvernalized winter and biennial varieties fail to flower under long-day conditions because of the absence of gibberellins, and under short-day conditions, because of the absence of both gibberellins and anthesins.

4. The proposed hypothesis needs further experimental and theoretical support. However, some data are presently available which support this hypothesis: the formation of flower primordia in nonvernalized seedlings of biennial henbane, due to the action of grafted leaves of short-day tobacco grown under long-day conditions; the difference between long-day and short-day varieties in the inhibiting action of leaves which are placed on unfavorable day length; the differences between these varieties in the response to the spectral composition of light; the direct relationship between vegetative growth and flowering in long-day varieties, and the absence of this relationship in the short-day varieties; the stimulation of flowering in long-day varieties by sugars, and in short-day varieties by nitrogenous substances.

5. The concept of flowering hormones presupposes that the basis of growth and flowering processes is the general metabolism of plants, in which many compounds, including auxins, vitamins, and enzymes, take an active part. The flowering hormones themselves, while arising under certain conditions as products of metabolism, in turn affect the changes in metabolism preceding morphological processes.

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METHODS

APPARATUS WITH THERMISTOR DIFFERENTIAL CARBON DIOXIDE ANALYZER FOR STUDYING THE KINETICS OF THE INDUCTION PERIOD OF PHOTOSYNTHESIS

V. E. Semenenko

K. A. Timiriazev Institute of Plant Physiology, USSR Academy of Sciences, Moscow

One of the most important lines of research on photosynthesis at the present time is the investigation of the kinetics and interdependence of the various systems and reactions – in many cases fairly well studied already – which jointly comprises the process of assimilation of light energy as a result of the photochemical synthesis of organic matter. A close study of the kinetics of the various reactions involved in photosynthesis only became possible with the development of highly sensitive methods and instruments (highly sensitive gas analyzers, manometry, labeled atom method and chromatography, differential spectrophotometry, etc.). While static investigations mainly provide information as to what links (systems) comprise the apparatus (process) of photosynthesis, kinetic investigations can provide data which reveal the interdependence of these systems and reactions in the realization of the whole process of photosynthesis.

This mobilization of different systems to common interrelated work takes place whenever light falls on the chloroplast and photosyntheses begins, i.e. during the induction period of photosynthesis. Hence an investigation of the mechanism of the processes of the induction period can be a convenient means of studying the mechanism of photosynthesis as a whole, since it enables us to visualize the photosynthesis process in its inception period, when the various systems and reactions are being incorporated, i.e. in its analyzed form. Investigators who have

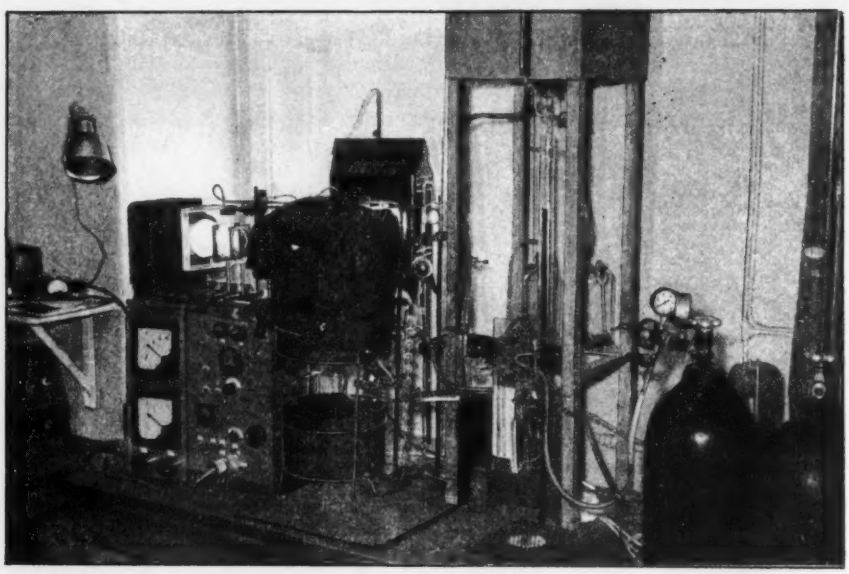

Fig. 1. General view of apparatus for studying the kinetics of the induction period of photosynthesis.

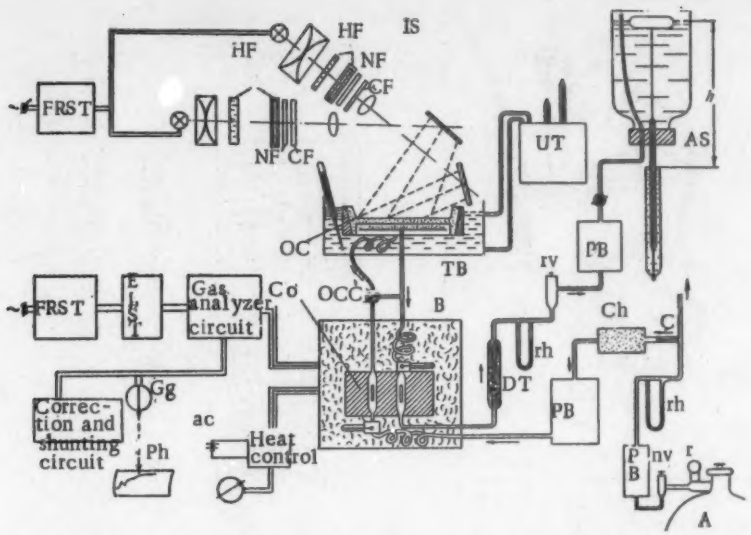

Fig. 2. Schematic diagram of apparatus for studying the kinetics of the induction period of photosynthesis.
A) Cylinder with fixed CO_2 content; r) reducer; nv) needle valve; PB) pressure box; rh) rheometer; c) capillary tubes; Ch) chamber for saturating gas with moisture (in bath TB with chamber OC); B) insulated bath for converter; st) spiral tubes; Co) converter; occ) cock for directing gas flow past object chamber; TB) thermostatted bath for chamber; OC) object chamber; UT) ultrathermostat; DT) tube with $CaCl_2$; As) aspirator; IS) illumination system; FRST) ferroresonance stabilizer; ELST) electronic stabilizer; Gg) galvanometer of gas analyzer; Ph) photorecorder; ac) accumulator. Arrows denote direction of gas flow in assembly.

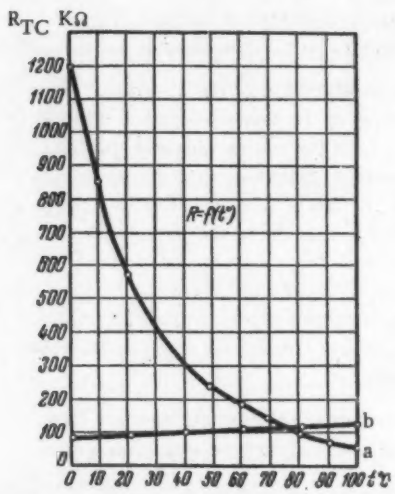

Fig. 3. Resistance of thermistor (a) and platinum (b) as a function of temperature [19].

succeeded in constructing sufficiently sensitive apparatus have established a number of specific features of the course of the induction period [1-10], which reveal important aspects of the internal mechanism of photosynthesis [11, 12, 13] and also indicate the complex processes involved in the "turning" of the photosynthetic apparatus at the onset of photosynthesis, when the chloroplasts acquire and later maintain the energy level and level of oxidation-reduction potentials which are necessary for the realization of photosynthesis [14].

Yet the nature and internal mechanism of the processes responsible for the complex behavior of the induction curves of photosynthesis still remain very obscure, although there have been several attempts (contradictory incidentally) to explain these processes [5, 6, 9 15]. Hence we undertook a study of the mechanism of the processes of the induction period of photosynthesis in the hope that a study of the conditions and factors which determine the absorption of carbon dioxide at different phases of the induction period would provide important information about the internal mechanism of photosynthesis.

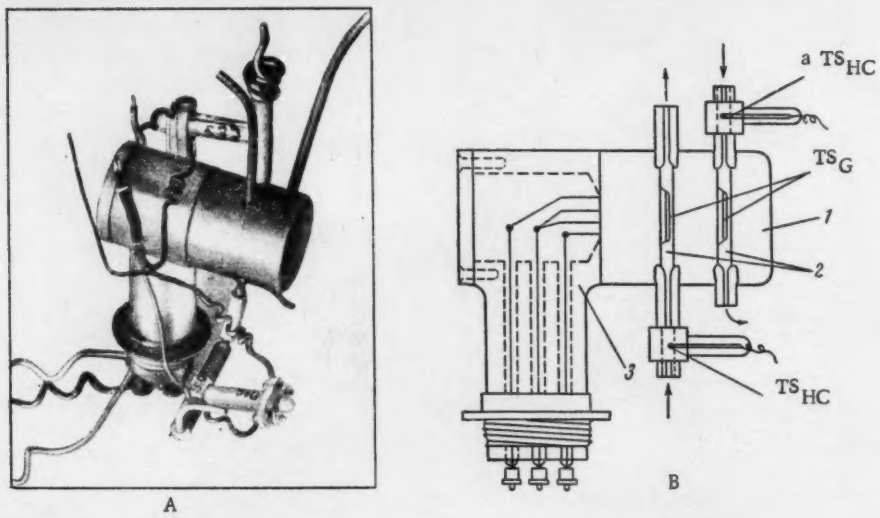

Fig. 4. Converter.
A) General view; B) schematic; 1) converter container; 2) tubes for gas flow; 3) base; TS$_G$) thermistors of gas analyzer; TS$_{HC}$) heat control thermistors. Arrows denote direction of gas flow in converter.

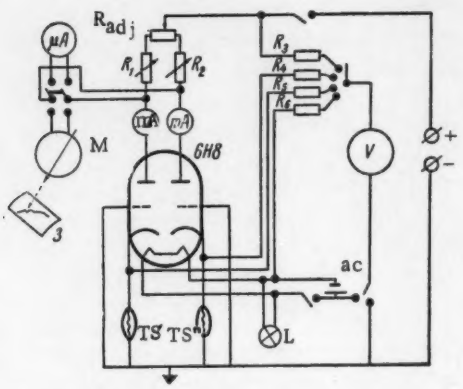

Fig. 5. Circuit diagram of gas analyzer.
TS' and TS") Gas analyzer thermistors; R$_{adj}$) wire resistance 1 kΩ; R$_1$ and R$_2$) wire resistances, each 5 kΩ; R) 2 meg; R$_4$ and R$_5$) 100 kΩ; R$_6$) 50 kΩ; M) mirror galvanometer; ph) photorecorder; ac) accumulator; L)indicator lamp.

For this purpose it was essential to construct a special apparatus provided with a highly sensitive continuous-recording gas analyzer, a special specimen chamber, illumination systems, etc.

A description of this apparatus is the subject of the present paper.

The apparatus (Fig. 1) consists of the following principal parts: 1) a highly sensitive continuous recording thermistor gas analyzer; 2) a system providing a flow of gas; 3) a system of thermal control; 4) a specimen chamber; 5) two illumination systems.

The basic arrangement of the apparatus for recording the kinetics of the induction period (Fig. 2) is the insertion of two converting elements (which measure the change in carbon dioxide content of the gas flow) in the gas flow before and after its passage through the chamber containing the photosynthesizing material. The converting elements are connected so as to measure the difference in CO_2 content before and after the object chamber according to a differential scheme, and this effect is recorded on a moving film. Thus, the principal component of the apparatus is a differential gas analyzer.

In selecting the type of gas analyzer we settled on the well-known principle [16] of utilizing the different thermal conductivity of gases, an effect which has been previously employed for studying the induction period of photosynthesis [4, 5, 9, 15] and which makes it relatively easy to attain an adequately sensitive apparatus with a quick response. The principle involved here is that the change in CO_2 content of the gas mixture of air can be measured by the change of thermal conductivity, which depends on the carbon dioxide content. This is made

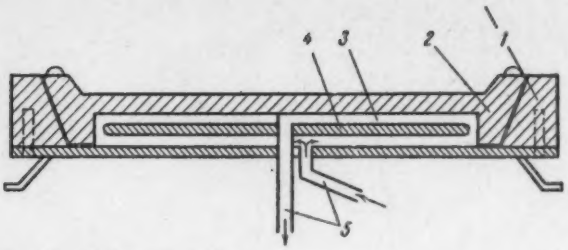

Fig. 6. Object chamber.
1) Body of chamber; 2) cover with window for light; 3) film of algae or leaf; 4) stage for object; 5) tubes for inflow and outflow of gas. Arrows denote flow of gas in chamber.

possible by the fact that the thermal conductivity of CO_2 differs significantly* from the conductivity of the principal components of the air as a whole, and thus permits the measurement of very small changes in the carbon dioxide content. If a current passes through an element heated to a definite temperature and situated in a flow of gas, and if the resistance of this element changes with temperature, the amount of heat dissipated by it, its temperature and, hence, its resistance will be constant so long as the composition, rate of flow, and temperature of the gas remain constant. If the composition of the gas changes (temperature and flow rate being constant), then the resistance of the element will also change in accordance with the dependence indicated, and this change can be recorded by electrical instruments.

Metal wires (most often platinum) are usually employed for such a measuring element. However, the temperature coefficient of their electrical conductivity is very small (0.3% per 1 deg). Hence we used for our conversion element the recently developed special heat-sensitive resistances (thermistors TS) [18-20]. As compared with metals they possess much higher temperature coefficients of resistance (α): a change of temperature of 1° alters the resistance by 2.7 - 6% (Fig. 3).

If two such elements are incorporated in a bridge circuit, one in the gas flow before the object chamber and the other after it, we obtain a converter of a differential gas analyzer, which will give a continuous record of the kinetics of the carbon dioxide content.

Thus, the main component of the gas analyzer is a converter (Fig. 4), in which a nonelectric magnitude (CO_2 concentration) is converted into an electric magnitude. The converter consists of a bulky copper container which carries two silvered tubes 4 mm in diameter. The gas passes through one of these tubes before it enters the object chamber, and leaves the chamber through the second. The tubes carry small thermistors (type MMT = 6, R_{20} = 9.5 kΩ, α = 3.2), which are connected in a tube bridge circuit and are in a heated state when the instrument is operating**. It is important here that the clearance between the TS and the tube walls is small (1-1.5 mm), since otherwise there will arise convection currents which will lead to serious error and loss of sensitivity. The converter is installed in a bath insulated from external fluctuations of temperature by asbestos and cotton wool (Fig. 2).

The tube bridge (Fig. 5) is assembled on a double triode 6H8C, to which the converter TS is connected as the cathode load, and the bias is applied to the grid. Thus each half of the tube with the cathode load (TS) forms two arms of the bridge, while the anode loads of the tube, composed of wire resistances together with portions of a variable resistance (R_{adj}), represent the second two arms of the bridge. The measuring instrument (wire-braced mirror galvanometer, sensitivity 10^{-8} A) is connected between the anodes of the tube and measures the unbalance which results from the change in resistance of the cathode load (TS), the reason for which, as we see ultimately, is the change of CO_2 content. In addition, the circuit contains a milliammeter and voltmeter which enable us to maintain the supply to the TS at one point of the volt – ampere characteristic and thus to obtain one temperature for the heating of the converter TS. Adjusting to zero is effected by means of a variable wire resistance R_{adj}. The power is supplied through a ferroresonance and electronic stabilizer. The tube filament is fed from an accumulator.

However, the basic scheme of the apparatus was complicated because, as we noted above, an essential condition for operation of the gas analyzer is the constancy of all other factors except the change in CO_2 content,

* Thermal conductivity $\lambda \frac{\text{in kcal}}{\text{m hr deg}}$: nitrogen – 0.01962, oxygen – 0.02005, air – 0.01919, carbon dioxide – 0.01179 [17].

** In selecting the pair of thermistors and the operating conditions it is important to determine their volt-ampere characteristics and to select each thermistor individually [18-23].

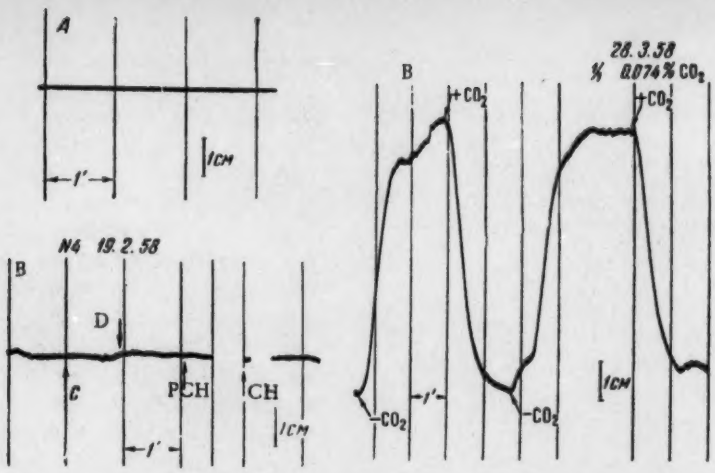

Fig. 7. Operating stability of apparatus and its sensitivity to CO_2.
A) Stability of operation of galvanometer; B) stability of operation of apparatus; C) chamber illuminated; D) illumination switched off; PCH) gas flow directed past chamber; Ch) gas flow directed through chamber; S) sensitivity of apparatus to CO_2 (change of CO_2 content in 0.074% at full sensitivity of galvanometer).

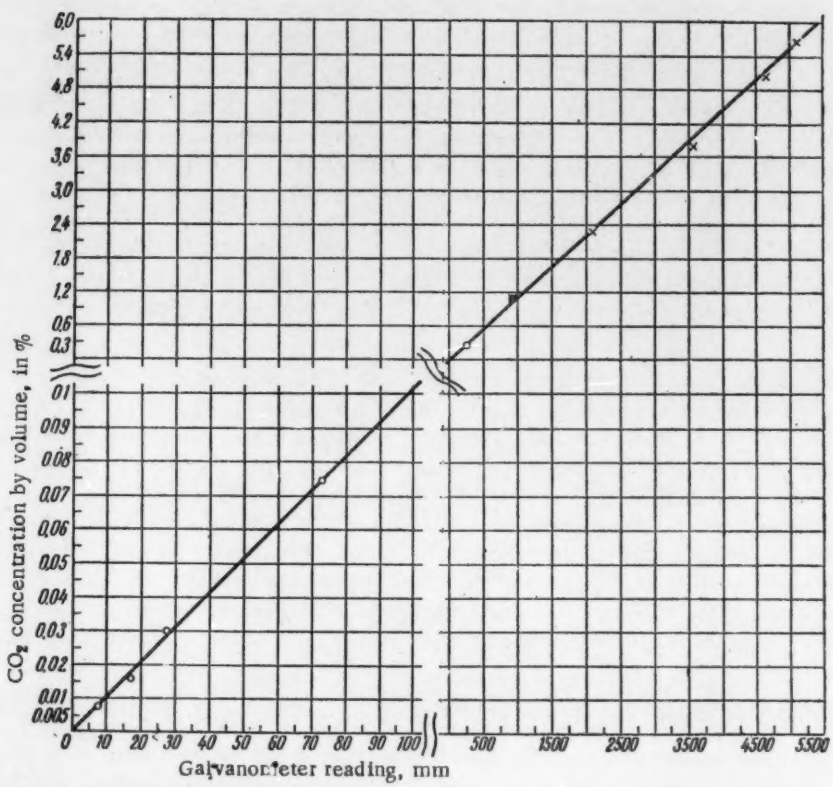

Fig. 8. Calibration curve.
O–o– calibration by Warburg buffers; –+–+ calibration by prepared mixtures of CO_2.

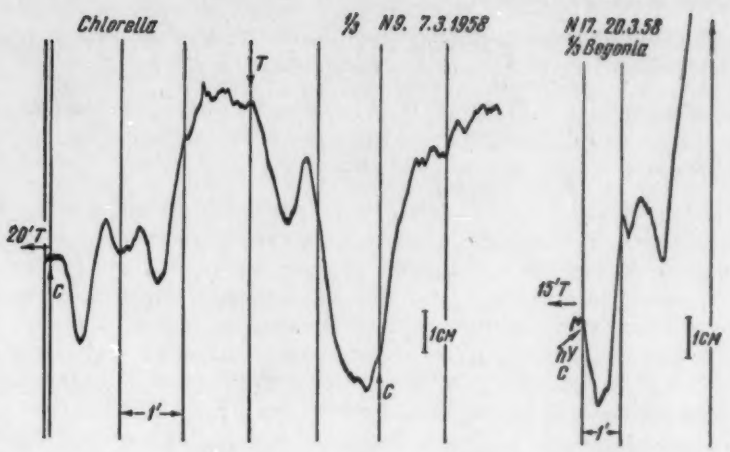

Fig. 9. Curves of kinetics of induction period of photosynthesis in Chlorella and Begonia.
C) Light switched on; D) light switched off. In experiment with Chlorella, temperature was 24°; light intensity 40,694 erg/cm sec; CO_2 concentration – 1.27%. In experiment with Begonia, temperature 22°; light intensity 40,694 erg/cm sec; CO_2 concentration – 3.48%.

which may also lead to a change in the amount of heat dissipated from the surface of the TS. These factors are changes in 1) air humidity; 2) temperature; 3) rate of gas flow; 4) other components of the air.

Hence it was necessary to eliminate the effects of all these factors on the converter of the gas analyzer, i.e. it was necessary to ensure that the humidity, temperature and flow rate of gas passing over first TS (before object chamber) and second TS (after object chamber) should always be the same.

This was achieved in the construction of the gas flow system of the apparatus. The gas flow system was basically the same as that used by Aufdemgarten [4], the only difference being that the humidity of the air was kept constant, not by drying as Aufdemgarten did [4], but by saturating with moisture, as Veen did, at the same temperature as that of the object chamber.

For maintaining a constant temperature of the gas falling on the converter TS, copper spirals were introduced into the gas flow system before the converter. In addition, the equality of the temperature of the gas falling on the first and second TS of the converter was subject to constant control. For this purpose, besides the two converter TS operating in the measurement of CO_2 concentration (in heated state), the gas flow system (before its entry into the converter tubes) was furnished with another two needle TS (made by the Agrophysical Institute), operating in the measurement of temperature. These TS were also connected in a bridge circuit (usual 4-armed bridge) and ensured that the gas bathing the converter TS was at the same temperature in each case. As a meter in the heat control bridge we used a zero galvanometer with a sensitivity ten times greater than the galvanometer of the gas analyzer (10^{-9} A).

The change of oxygen content as a result of photosynthesis did not affect the reading of such a gas analyzer since the thermal conductivity of oxygen is very close to that of air, and its percentage content in the air is so high in comparison with the changes resulting from photosynthesis that it does not interfere in the work. For ensuring an even flow of gas into the apparatus there was a system (Fig. 2) of pressure boxes, capillaries, needle valves and rheometers, and for drawing the gas through the apparatus we used a well-known type of aspirator operating under a constant acting force h – the difference between the upper level and the exit level of the liquid. The rate of gas flow could be regulated within fixed limits and for our work was set at 80 ml/min.

The gas was drawn into the apparatus through a capillary from the tube it passed through after it left (at a speed of 120 ml/min) the cylinder containing a prepared mixture of fixed CO_2 content.

The gas flow tubes between the chamber and converter were made from millemeter capillary in order to ensure the quickest response of the apparatus.

The flow system was furnished with a connecting tube by which the gas flow could be directed past the chamber, and thus permit a measurement of the effect of respiration of the object.

An important part of the apparatus was the special object chamber (algae or higher plant) which could accommodate a fairly large area of algal film or leaf, but had a very small volume (an essential feature for reducing the time lag of the apparatus).

The round chamber (Fig. 6), made from plexiglas, diameter 10 cm, had a ground cover, which also served as a window for transmitting light. Beneath the cover there was a stage which was mounted in such a way that there was a clearance of 1 mm between the side, bottom and top of the chamber. The gas entering the chamber through an opening in the center under the stage, left it through a second opening in the center of the stage, and thus bathed the object on the stage with radially propagated currents of gas in every direction. The diameter and height of the chamber could be altered by means of special fit-on rings and a set of stages which enabled us to alter the speed of response of the apparatus on the one hand, and on the other, to select the optimal area of the object for obtaining distinct traces of the kinetics of the induction period. The chamber was placed in a bath over the converter under a layer of flowing water thermostatted in an ultrathermostat (Fig. 2).

The apparatus was equipped with two lighting systems which enabled us to illuminate the object with light of various intensities and spectral composition. For the infrared region of the spectrum we used cuvettes with flowing water and a cuvette which contained a glass heat filter (SZS-14), also cooled by a current of water. For changing the intensity of the light we employed neutral glass filters NF, and for obtaining light of fixed spectral composition — color filters (SS-5, KS-2, ZS-1, etc.).

In addition there was a set of light sources consisting of 500 and 750 watt projection lamps, PRK lamps, mercury-cadmium and sodium lamps, which enabled us to vary the intensity and spectral composition of the light over a wide range.

A test of the assembled apparatus showed that it was perfectly suitable for the investigation of the questions contemplated.

The time lag of the apparatus was 8-12 sec with an object chamber of maximal volume.

As a check to ensure that the manipulation of the apparatus (illumination and darkening of object, passing the gas through the chamber or past it) did not give side effects, we recorded a trace of the zero line of the apparatus (Fig. 7, B). In this case a moist disk of green paper replaced the algal film in the chamber. In addition, we had to use a special wire-braced galvanometer which was not disturbed by vibration, since this factor alone can very seriously affect the operational stability of ordinary galvanometers (Fig. 7, A).

The instrument was calibrated in three different ways — by the method of parallel gas flows of different CO_2 content through both tubes of the converter, by absorption of CO_2 from the gas flow after its passage through the first tube of the converter by using prepared gas mixtures and various Warburg buffers. The results of calibration, given on the calibration curve (Fig. 8) and a photogram (Fig. 7, C) showed adequate sensitivity and linearity over a range much greater than that in which induction phenomena operate.*

The sensitivity was found to be higher than that necessary for recording the kinetics of the induction period of photosynthesis. In order to obtain complete photograms, it was necessary to shunt the galvanometer by a factor of 5 or 10, or even more. This meant that we could decrease the area of the object, and hence the volume of the chamber, and thus reduce the time lag of the apparatus.

On Fig. 9 we give examples of photographic traces of the kinetics of the induction period, obtained by means of the described apparatus. As we see from these photograms, the obtained curves are completely in agreement with the facts previously obtained by other authors and reveal certain new details which are also subject to interpretation on the basis of the already known laws.

* Nonlinear response began with the measurement of CO_2 concentration of the order 5%, when the data obtained were approximately 0.005% below the true value.

The apparatus may be considerably simplified if it is to be used for less special purposes.

In conclusion, I express my sincere thanks to my scientific chief Prof. A. A. Nichiporovich. I take the opportunity also of expressing my thanks to radio technician V. P. Kornil'ev for help in constructing the apparatus and to M. P. Vlasova for help with the calibration.

While working on the construction of this apparatus I derived constant inspiration from the memory of my father Efim Semenoviche Semenenko, who taught me to love work.

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BOOK REVIEW

A PRACTICAL MANUAL OF PLANT PHYSIOLOGY

Nauka i Izkustov, Sofia, 1957

K. Iord Popov, G. Khr. Georgiev, R. Docheva-Popova, and G. K. Gushcherov

The teaching of plant physiology as an experimental science impels universities to use two books simultaneously: a text book for the theoretical courses of study and an educational accessory — a practical manual. It is to be regretted that each type of book is ordinarily compiled by a different author, and for this reason alone the organic unity of the subject matter is disturbed. It is comforting to note that the plant physiologists of Sofia University managed to overcome these difficulties. Prof. Kiril (Cyril) Iordanov Popov, who compiled a course of study in plant physiology in Bulgarian (1956), has also compiled a practical manual with his collaborators (1957).

In P. A. Genkel's review, published in the journal "Plant Physiology", Volume 4, No. 3, the attention of the Soviet reader was already directed to the merits of the first work. Therefore we shall confine our remarks in this case only to the second book. This is a practical manual in physiology for universities. The manual is of considerable size. Due to compact presentation, wide use of brevier *, and clear illustrations, the authors found it possible to describe 329 experiments on 331 pages. The vast material is well systematized and arranged in the following order: preface, introduction, and such sections as the composition and structure of the vegetative cell (the chemical composition, basic properties of colloidal solutions), absorption and liberation of substances by the vegetative cell (absorption of water by the cell, cell penetrability), enzymes and vitamins, water cycle of plants (the soil as water source, absorption of water by roots, transpiration, movement and ascent of water in the plant, water balance, drought-resistance), photosynthesis, chemosynthesis and bacterial photosynthesis, nitrogen nutrition (nitrogen nutrition of green plants, biological nitrogen-fixation, heterotrophic plant nutrition), assimilation of mineral elements, plant assimilants, fermentation and fermentation microorganisms, respiration, seed germination and bud development, growth (growth measurements and localization, effect of external environments on growth, growth activators, correlation and polarity), regeneration and transplantation, plant development, stimulation and motion in plants (tropisms, plastic motion, taxis), sexual reproduction. The manual concludes with the principles of microbiological technique. Apart from referring to original sources when individual experiments are described, the authors furnish at the end of the book a complete list of literature references.

In their manual the authors make wide use of both foreign and Russian, especially Soviet literature on plant physiology methods. The size, and to a certain degree, also the organization of the manual under review reminds one of the manuals by Skazkin et al. (1953) and Valter and Pinevich (1957), well known to Soviet readers. At the same time we have before us an original educational manual. It should be noted first that the authors introduce a number of new problems, borrowed not from textbooks but from the current scientific literature, as well as from their own experiments (determinations of coagulative temperature threshold according to Genkel', determination of transpiration according to Arland, the qualitative detection of photosynthetic nitrogenous products according to Chailakhian, seed stimulation by the M. Popov method, and others).

The authors should also be given credit for the fact that all sections of the theoretical course of study find corresponding material in the manual, in which such problems are also fully represented that normally are either undeservedly omitted in practical manuals on physiology or are presented in a very abridged form: tropisms,

* An 8-point type.

nastins, taxis, correlations, polarity. The methodological qualities should be given special notice in the manual. In particular, the authors consistently maintain this principle: instructions proceed from the simple to the complex. Within each new section, as a rule, at first model experiments are described which explain the fundamentals of the newly studied process; then attention is focused on methods of qualitative determination, and, finally, a series of experiments is suggested illustrating a number of theoretical processes, very frequently the effect of external factors on one or another physiological process (for instance, for photosynthesis — effect of light power, different regions of the spectrum, temperature, CO_2 content). And, finally, the authors deserve recognition for the abundance of schematic drawings and illustrations (over 200) which supplement the descriptions of experiments very well.

The manual is compiled, as stated earlier, for universities. However, the authors took into account some wider demands as well, realizing that Bulgarian universities train a considerable number of students as teachers of middle schools. This could not but reflect on the manual's content. The manual is somewhat over-filled with elementary studies and, at the same time, abridged by elimination of more complex problems, such as those requiring special appliances. For example, no problems are discussed which relate to the use of special appliances for the microscope: ocular micrometer, opaque illuminator, a dark-field condenser, etc.; chromatographic methods are insufficiently discussed: no problems are undertaken on separation of leaf pigments by the M. S. Tsvet method; no indications are present on utilization of ion-exchange resins; the omission in the manual of some problems which have rightfully become classic is to be regretted, such as the electrometric method of determining pH, manometric methods for determining respiration and photosynthesis, determination of individual carbohydrate forms in a common sample.

All this, of course, diminishes the merit of the book reviewed as a contemporary educational accessory for universities, but the book is rather valuable in many other ways. It is our deep conviction that the manual will find wide application. The enumerated faults notwithstanding, it still remains an original university manual on plant physiology; secondly, due to methodological merits (presentation in a popular and brief form, orderly arrangement of material, organic relationship with a theoretical course of study), the book will be of great assistance to independent study, and therefore will find its niche in the system of educational correspondence courses; thirdly, it will play an important part in teaching plant physiology in middle schools where, by the way, "Plant Life in Experiments" should occupy a special position in relation to polytechnization; finally, it may become a valuable auxiliary in demonstrating lecture courses on plant physiology in universities.

On the whole, the book reviewed is a worthy continuation of the successfully compiled text book on plant physiology by K. Popov. This success will bring joy to Bulgarian and Soviet students. We express hope for a speedy translation of this, in many respects excellent book, into Russian.

P. S. Belikov
I. T. Iordanov

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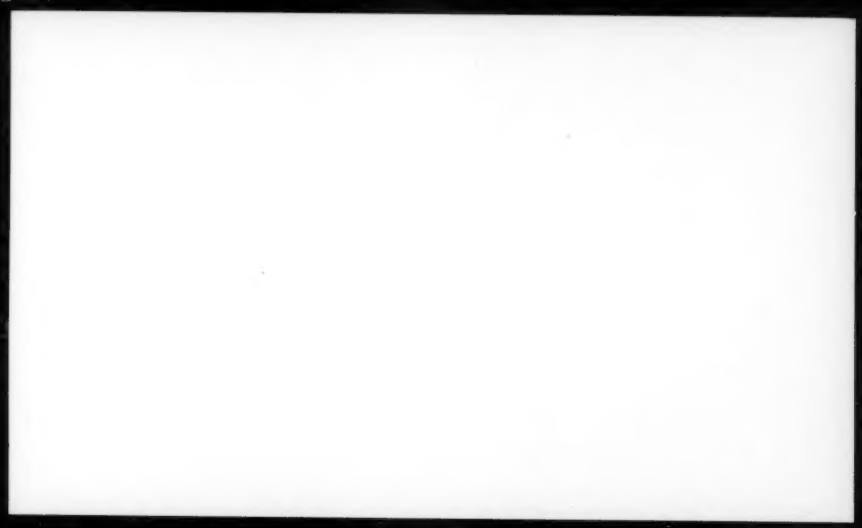


ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

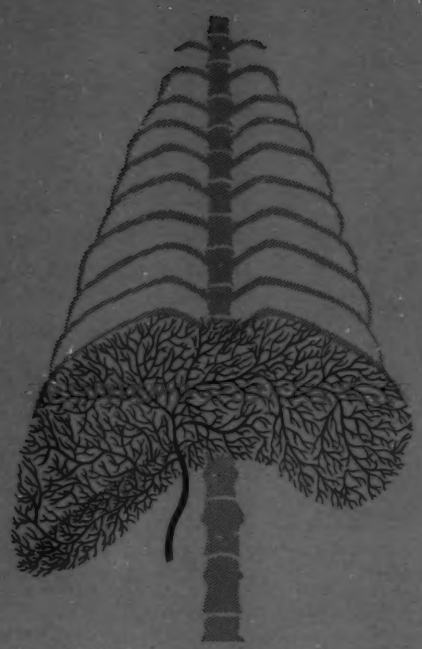
Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspti. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology



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The AIBS is in the process of expanding its Russian Translations Program extensively. Funds to subsidize translation and publication of important Russian literature in biology have been obtained from the National Science Foundation, as part of a larger program to encourage the exchange of scientific information between the two countries. The following monographs have been scheduled for early publication:

Origins of Angiospermous Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. 68 pgs. Ready now. $3.00 (U.S. & Canada) $3.50 (Foreign)

Essays on the Evolutionary Morphology of Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. Ready November 1958. $5.00 (U.S. & Canada) $5.50 (Foreign)

Problems in the Classification of Antagonists of Actinomycetes. By G. F. Gause. Edited by David Gottlieb. Translated by Fritz Danga. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

X-Rays and Plants. By L. P. Breslavets. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

Arachnida. Vol. IV, No. 2. Fauna of the U.S.S.R. By B. I. Pomerantzev. Edited by George Anastos. Translated by Alena Elbl. Ready early 1959. $10.00 (U.S. & Canada) $11.00 (Foreign)

Arachnoidea. Vol. VI, No. 1. Fauna of the U.S.S.R. By A. A. Zachvatkin. Translated and edited by A. Ratcliffe and A. M. Hughes. Ready spring 1959. $10.00 (U.S. & Canada) $11.00 (Foreign)

Three new Russian journals are being added to the list of four currently translated and published by AIBS:

Soil Science (Pochvovedenie). 12 issues per year. Approx. 1,600 pgs. per year. Will begin with January 1958 issue. Ready January 1959.

Subscriptions: $40.00 per year (General)
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Entomological Revue (Entomologicheskoe Obozrenie). 4 issues per year. Approx. 250 pgs. per year. Will begin with January 1958 issue. Ready January 1959.

Subscriptions: $25.00 per year (General)
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$3.00 additional (to each price) for foreign orders
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Doklady—Biochemistry Section. 6 issues per year. Approx. 500 pgs. per year. Currently being translated and published by Consultants Bureau, this section of Doklady will now be published by AIBS, beginning with January 1958 issue. Ready September 1958.

Subscriptions: $15.00 per year (General)
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